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VOLUME 39 OCTOBER 1961 NUMBER 5

APHELENCHOIDES SUBPARIETINUS N. SP. (NEMATODA: APHELENCHOIDIDAE) FROM DISEASED LILY BULBS[1]

K. C. SANWAL

Abstract

Aphelenchoides subparietinus n. sp. collected from diseased bulbs is described and figured. The new species is closely related to and is compared with *A. parietinus* s. str. (Bastian, 1865) Steiner, 1932; *A. saprophilus* Franklin, 1957; *A. composticola* Franklin, 1957; *A. cyrtus* Paesler, 1958; *A. sacchari* Hooper, 1958; and *A. helophilus* (de Man, 1880) T. Goodey, 1933.

Forty-one nematodes (26 females, 15 males) and a large number of juveniles were collected from diseased lily bulbs imported from a commercial seed firm from the United States, and intercepted by the Plant Protection Division of the Canada Department of Agriculture, Ottawa. This population consisted of seven females and five males of an unidentified species of the genus *Seinura* Fuchs, 1931 (5), six females of *Aphelenchoides parietinus* s. str. (Bastian, 1865) Steiner, 1932 (1, 10), and 13 females and 10 males of a new species of *Aphelenchoides* which is described below. More than half the specimens were studied while living.

Aphelenchoides subparietinus n. sp.

Female (n = 11).—Length = 0.58–0.69 mm; a = 29.8–31; b = 9.3–10.9; c = 11.9–14.5; V = 63.5–68%.

Body small and transparent, not markedly attenuated in anterior region, but rather sharply attenuated posterior to anus. Cuticle with very fine transverse striations. Lateral fields and incisures absent. Body lies straight with only slight curvature of tail region (assuming shape of a hockey-stick), when relaxed by gentle heat (Fig. 1).

Head distinctly set off from body. Face view of head shows six lips but papillae not visible. Stylet 11–12 μ long with basal thickening, but without well-defined knobs as in *A. ritzemabosi*. Dorsal basal thickening of spear apparently larger than subventral thickenings. Precorpus narrow, followed by a prominent median bulb. Median bulb slightly longer than broad, in

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Contribution from the Nematology Section, Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.

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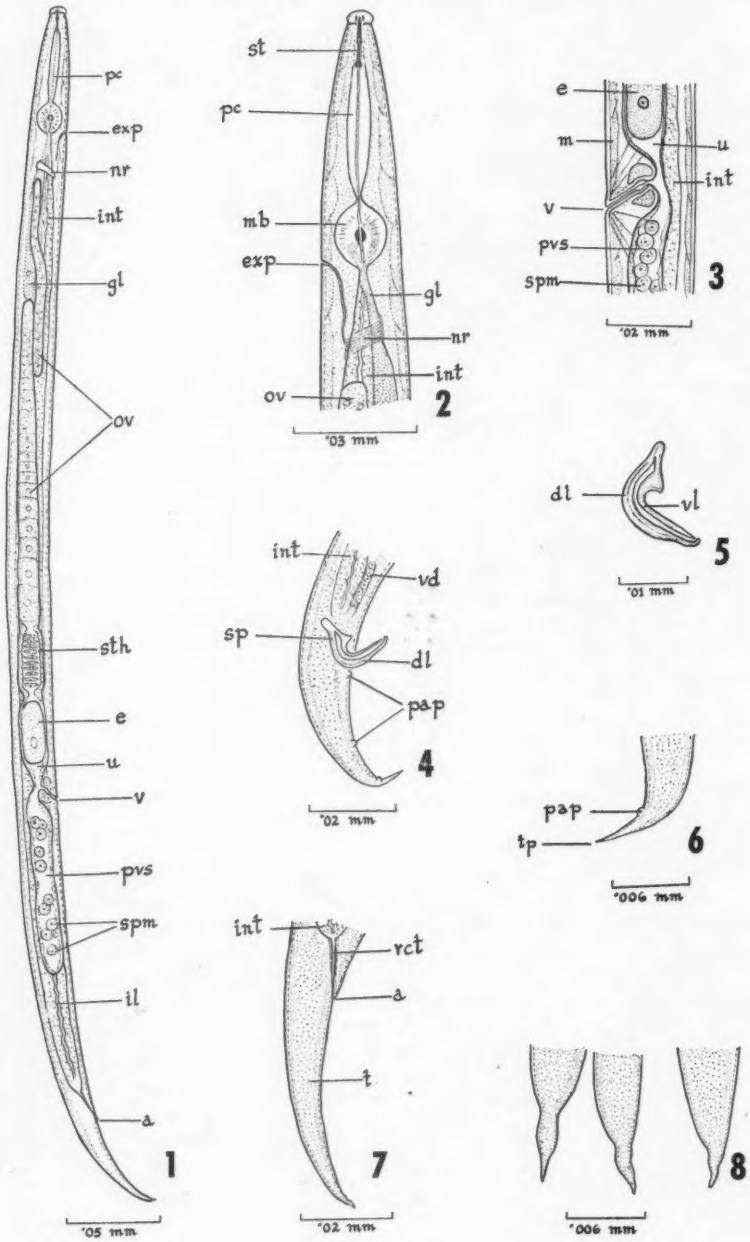
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some specimens almost spherical, and occupies two-thirds of body width. Very narrow constriction at junction of precorpus and median bulb. Bean-shaped crescentic thickenings of lumen of median bulb conspicuous. Esophageal glands lie dorsal to intestine, which begins immediately behind median esophageal bulb (Fig. 2). Rectum long and narrow.

Nerve ring surrounds intestine and esophageal glands about one body width posterior to median bulb. Excretory pore opens ventrally at level of posterior margin of median esophageal bulb in majority of specimens but in some it is situated a little behind the posterior margin of bulb. In all cases, however, it is located anterior to nerve ring (Fig. 2).

Vulva situated at approximately two-thirds of body length from anterior end (Figs. 1, 3). Vagina directed anteriad. Uterus in most cases contained one large egg. Ovary single, very long, outstretched, extending always beyond posterior margin of esophageal glands, almost to nerve ring or even beyond median esophageal bulb in some specimens. Ovary with one or two U-shaped or S-shaped loops. Terminal end of ovary may or may not be reflexed. Spermatheca about 1–2 times body width in length, filled (in most cases) with disklike sperms. Postvulvar uterine sac well developed, 4–5 times body width in length, filled with globular sperms. Ratio between length and breadth of egg in uterus 2.6–3.4:1.

Rather long, slender conical tail, with body narrowing sharply beyond anus. Tail 4–5 anal diameters long, with a well-defined steeple-shaped termination (Figs. 1, 7, 8). (The slender tail never lies flat on the surface of the slide beneath a cover slip and this may result in completely different appearances of the tail shape. Placing the nematode beneath a cover slip without glass rods is helpful in studying the shape of the tail tip.) Shape of tail tip in female varies to some extent (Fig. 8).

Male (n = 9).—Length = 0.51–0.58 mm; a = 26.9–29.4; b = 8.0–9.6; c = 12.7–14.4.

Cuticle with fine transverse striae. Lateral fields absent. Head, lips, stylet, esophagus, esophageal glands, position of nerve ring, and excretory pore same as in female. Intestine begins immediately behind median esophageal bulb. Stylet 10–11 μ in length.

Single testis, extending anteriorly a little beyond the posterior margin of esophageal glands. In some specimens, however, it may extend further to a point beyond the posterior margin of median esophageal bulb. Two spicules present, equal in size, typically aphelenchoid in shape, sharply arcuate, with a conspicuous ventral apex (Figs. 4, 5). Length of dorsal limb 0.023–0.024 mm; length of ventral limb 0.012–0.014 mm. Dorsal limb not smoothly curved but shows a slight depression in its outline towards tip.

FIGS. 1–8. *Aphelenchoides subparietinus* n. sp. Fig. 1. Female showing general anatomy. 2. Anterior region of female. 3. Vulvar region of female. 4. Tail region of male. 5. Spicule. 6. Tail tip of male. 7. Tail region of female. 8. Variation in the shape of female tail tip. *Abbreviations*: a, anus; dl, dorsal limb; e, egg; exp, excretory pore; gl, esophageal gland; il, intestinal lumen; int, intestine; m, muscle layer; mb, median bulb; nr, nerve ring; ov, ovary; pap, caudal papillae; pc, precorpus; pvs, postvulvar uterine sac; rct, rectum; sp, spicule; spm, spermatozoa; st, stylet; sth, spermatheca; t, tail; tp, tail tip; u, uterus; v, vulva; vl, ventral limb.

Tip of dorsal limb not sharply pointed and slightly curved inwards.The two pieces of the dorsal limb are heavily sclerotized and the hollowness of the limb is not apparent. Ventral limb almost reaching tip of dorsal limb although in processed specimens it may not appear so, because of the transparency of its tip region. Gubernaculum absent.

Tail about three anal diameters long with a sharply pointed and conspicuous spinelike termination which has a constant shape (Figs. 4, 5). Three pairs of postanal subventral papillae present: one pair adanal, one pair midway along the tail, and a third pair at base of spinelike termination.

Host.—Lily bulbs.

Locality.—From a commercial seed firm in Illinois, U.S.A.

Holotype (female) and paratypes deposited in Canadian National Collection of Nematodes, Nematology Section, Ottawa, Canada, collection No. 2161.

Relationships

Franklin (3) redescribed *Aphelenchoides parietinus* from the type locality. On the basis of this study she pointed out that some forms described by various authors as *A. parietinus* and also some existing synonyms of *A. parietinus* may represent species distinct from *A. parietinus*. After the availability of Franklin's description of *A. parietinus* s. str., it has been possible to distinguish between such species as *A. saprophilus* Franklin, 1957 (4), *A. composticola* Franklin, 1957 (4), *A. sacchari* Hooper, 1958 (7), and *A. kuehnii* Fischer, 1894 (2), which are closely related to *A. parietinus* but are morphologically distinct from it. *A. subparietinus* described above is another species which is closely related to *A. parietinus*, *A. saprophilus*, *A. composticola*, *A. cyrtus* Paesler, 1958 (9), *A. sacchari*, and *A. helophilus* (de Man, 1880) T. Goodey, 1933 (8, 6). The differences between *A. subparietinus* and these species are briefly outlined below.

Aphelenchoides subparietinus can be distinguished from *A. parietinus* s. str. by the absence of lateral fields, posterior position of the nerve ring in relation to the excretory pore, slenderer tail, different shape of male and female tail tip, shorter egg, longer postvulvar uterine sac, different curvature of female tail when relaxed by gentle heat, and different ratio between the length and width of the body.

Aphelenchoides subparietinus differs from *A. cyrtus* in having different body proportions and slenderer tail, in the different shape of male and female tail tip, in the position of excretory pore in relation to nerve ring, in the absence of lateral fields, in size and extent of ovary, in the shape and curvature of spicules, and in the curvature of the body which does not assume the shape of a bow when relaxed by gentle heat.

From *A. composticola* and *A. saprophilus* the new species differs in absence of lateral fields, in position of excretory pore in relation to nerve ring, in the shape of female tail, and the tip of male and female tails, in the size and extent of ovary, and in the sharper curvature of spicules.

From *A. sacchari*, this species can be differentiated by the shape and size of female tail, by the shape of male and female tail tip, by the absence of lateral fields, and by the different size and shape of spicules.

From *A. helophilus*, the new species can be distinguished by the smaller body size and different body proportions, by the less-pronounced basal thickening of stylet, by the different position of the excretory pore in relation to the nerve ring, and by the sharper curvature and smaller size of spicules.

The asymmetrical thickening of the base of the stylet has not been used in the differentiation of *A. subparietinus* from other species. This character was evident in all the specimens examined in the living condition. However, a special study was not made of this character and the probability of optical abberation cannot be completely ruled out. Asymmetrical basal knobs of the stylet, although not reported so far in members of Aphelenchoidea, are, however, a diagnostic character of the genus *Stictylus* of Neotylenchidae, and the possibility of their occurrence in other stylet-bearing nematodes is not untenable. The absence of lateral fields and lateral incisures in *A. subparietinus*, however, has been checked carefully by various microscopical techniques including phase contrast.

Acknowledgment

The author wishes to thank Dr. A. D. Baker, Chairman, Nematology Section, Entomology Research Institute, Ottawa, for valuable criticism in preparing the manuscript.

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THE LIFE HISTORY OF MOLINEUS BARBATUS CHANDLER, 1942[1]

S. P. GUPTA[2]

Abstract

Using the ferret as an experimental host, the life cycle of *Molineus barbatus* was shown to be direct. All stages are described. The third-stage larva infects the host orally or percutaneously and subsequent stages develop in the mucosa of the small intestine. The male matures earlier than the female and eggs are laid in 8 to 13 days after infection. The parasite is highly pathogenic to ferrets but apparently less so to skunks and racoons.

The genus *Molineus* was created in 1923 by Cameron (2) for a trichostrongyle worm from *Felis yaguarundi* from South America. Since then a considerable number of species have been recorded from most regions of the world, all (with the exception of two species which occur in South American monkeys and one in the African potto) from carnivores. The subject of the present study, *Molineus barbatus*, was described by Chandler in 1942 (3) from the racoon (*Procyon lotor*). It also occurs naturally in the skunk (1) in North America and it can be transferred to the ferret (*Putorius putorius domesticus*).

The donors in this study were two racoons,[3] the faeces from which were cultivated in petri plates with sterile sand and charcoal at room temperature (22° to 24° C) and the larvae recovered in 5 days by the Baermann technique. Skunks and ferrets were both infected with these larvae.

Experimental infections were carried out on 17 ferrets and two skunks. The first skunk died from a heavy mixed infection of the hookworm *Placoconus lotoris* and *Molineus barbatus*, showing that the infection in the original donors, the racoons, was a mixed one. Two of the earlier infections in ferrets were also mixed but subsequent infections were made by teasing up female *Molineus* from these ferrets and infecting others from this source. This technique was repeated subsequently when more pure cultures were required.

In ferrets the development was rapid, eggs being found in their faeces in from 8 to 10 days. However, the duration of the infection was short and the mature worms appeared to live for only a few days. To make a complete study of the life cycle of the parasite, therefore, a series of ferrets were infected and individuals were killed after 2, 3, 5, 7, 8, 12, and 13 days. On the second day a few larvae were present in the stomach but, thereafter, the parasite was recorded mainly from the anterior third of the small intestine, although a few larvae were present in the middle and terminal thirds. After the fifth day, most worms were partly or completely embedded in the mucosa of the small

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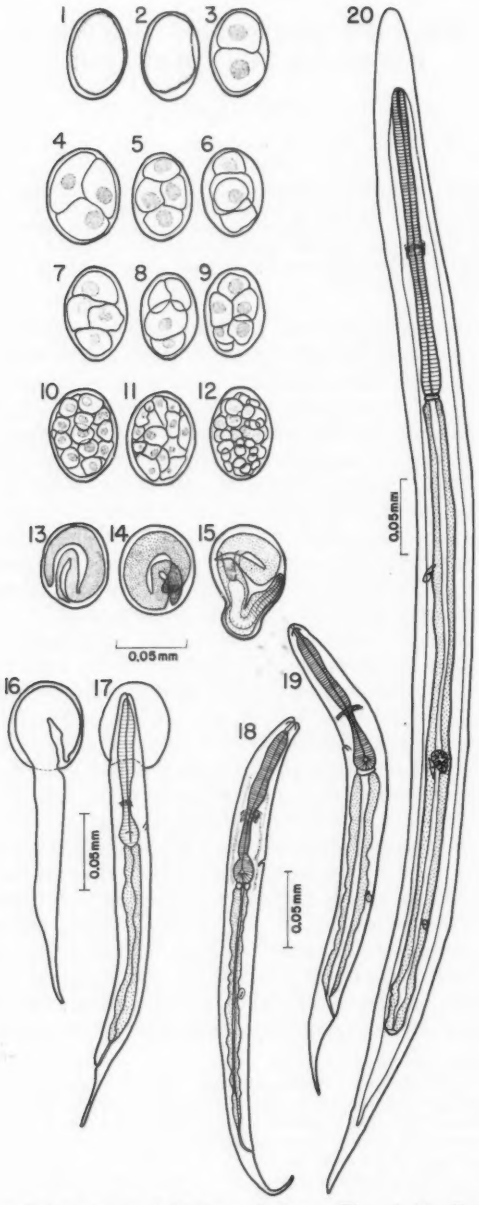
[2]Postdoctorate Fellow, National Research Council of Canada. Present address: Department of Zoology, University of Lucknow, Lucknow, India.

[3]One of the racoons was shot locally by Dr. G. A. Schad, the other was obtained from Dr. Carlton M. Hermann, Chief, Section of Wildlife Diseases and Parasitic Studies of the U.S. Department of the Interior. To both of these, the author owes his thanks.



FIGS. 1–20. Free-living stages of *Molineus barbatus*. Figs. 1–15. Embryonating egg. Figs. 16, 17. Hatching of the egg. Fig. 18. First-stage larva. Fig. 19. Second-stage larva. Fig. 20. Third-stage or infective larva.

intestine and when the infections were heavy caused a haemorrhagic enteritis (Fig. 53). Worms were recovered from this source by pepsin digestion, as described by Herlich (5).

All morphological studies were made on material fixed in hot 70% alcohol and cleared in glycerin, or on unfixed larvae after staining with iodine, neutral red, methylene blue, or methylene green. All drawings were made with the aid of a camera lucida.

Description

Eggs (Figs. 1–17)

The eggs are typical, thin-shelled trichostrongyle eggs, measuring 54–61 μ long by 38–45 μ broad. The ovum has commenced morulation when passed from the female but subsequent embryonation occurs outside the body of the host. Its rate is determined by the ambient temperature but there is no development under 5° C and the embryos do not survive at 40° C or over. Observed in shallow water in petri dishes, the first larval stage was reached and hatching took place in about half of the eggs under observation in 8 days at 10° C, 4–5 days at 15° C, 25 hours at 20° C, 22 hours at 25° C, and 17–18 hours at 30° C and 35° C.

The First-stage Larva (Fig. 18)

The newly hatched larvae move very slowly at first but as soon as feeding begins they become active and remain so until the first lethargus, which occurs some 22 hours after hatching at 22° to 24° C.

The larvae measure from 0.23 to 0.27 mm in length—an average of 25 specimens was 0.26 mm—with a width of 0.015 mm. There is a cylindrical mouth tube, 6 μ long, which opens into a rhabditiform oesophagus 0.07 mm long. The intestine, which is composed of 14–16 cells, opens at the rectum, 0.04 mm from the posterior tip of the body. The nerve ring surrounds the isthmus of the oesophagus and contains a number of nuclei of nerve cells. The excretory pore opens on the ventral side of the body a short distance posterior to the nerve ring.

The genital primordium is a small elliptical body lying ventral and posterior to the sixth intestinal cell. Four coelomocytes are present—one associated with the genital primordium, one immediately behind the oesophagus, and the remaining two about equidistant on each side of the genital primordium.

The Second-stage Larva (Fig. 19)

The first ecdysis occurs at a temperature of 22° to 24° C some 43 hours after hatching. The second-stage larva, on escaping from the cast cuticle, is 0.30 mm long, but within 12 hours it has grown to 0.38 mm long and, by the time of the second lethargus, it is 0.57 mm long. It does not differ materially from the first stage but as it grows the tail becomes correspondingly longer and more slender, and the cells of the genital primordium are increased to 6–8.

The Third-stage Larva (Fig. 20)

At a temperature of 22° to 24° C the second ecdysis is reached about 90 hours after hatching, but the third-stage larva is slightly smaller than the fully developed second stage, measuring 0.52 mm. It is, however, contained

within the cast cuticle of the second stage (which measures up to 0.59 mm). It is more slender than the previous stage and both it and the sheath appear finely striated. The mouth tube has now disappeared, and the oesophageal bulb has lost its valvular apparatus and has become more slender. The tail is conical and shorter, ending in a characteristic digitiform process.

The nerve ring surrounds the mid-point of the oesophagus and in stained specimens 6 papillary nerves are represented anterior to it by a chain of nuclei while the primordia of a number of cephalic ganglia lie just posterior to it.

The genital primordia are of two types. In the males, the primordium is oval or round with 9–11 epithelial cells and 2 germinal cells lying at the posterior part of the rudiment. In the females, there are 7–8 epithelial cells and the primordium is elliptical with a germinal cell at each end; one epithelial cell is polar while the others are symmetrically arranged on each side.

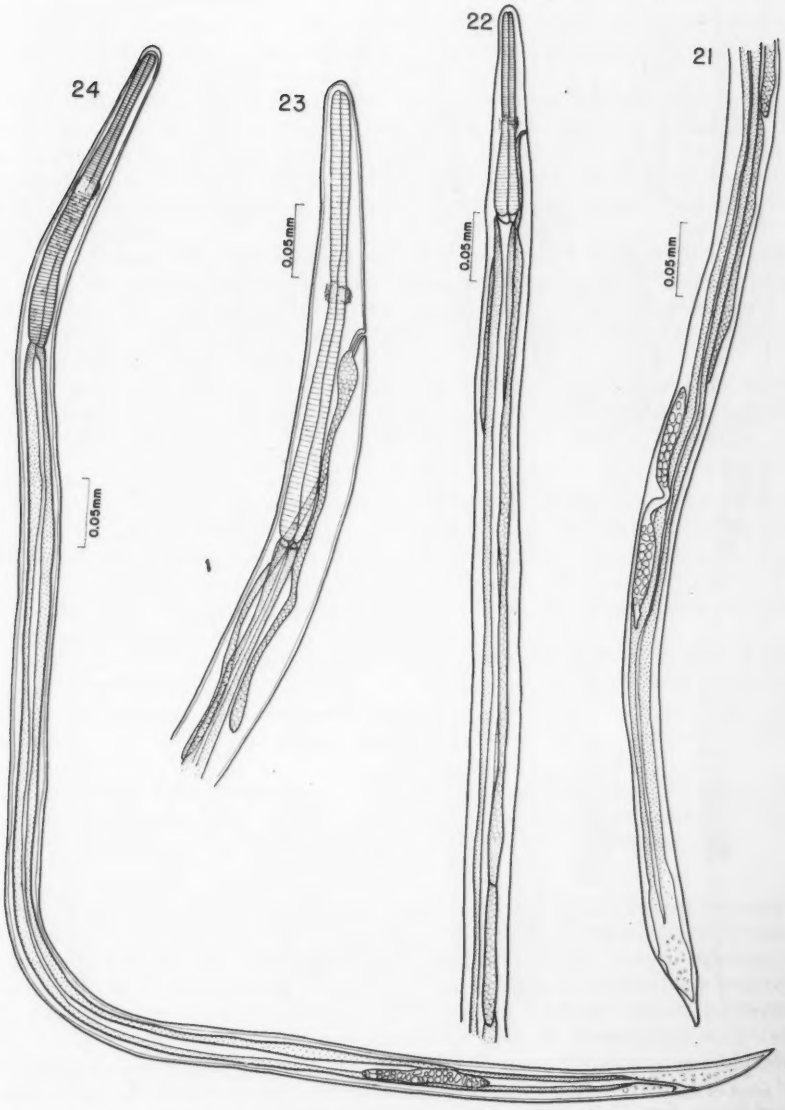
The infective larvae have a life of over 3 months in tap water at temperatures of 15° C and under. At 20° C, however, half died in 15 days while at 25° C two-thirds were dead in this time; a few at both temperatures lived for 3 months. At 30° C all were dead in 47 days, at 35° C all in 38 days; and at 40° C only a few survived for 10 days, and all were dead in 18 days.

Using the usual laboratory techniques, it was shown that the infective larvae could not withstand even brief periods of desiccation but that they could leave a moist, semisolid culture in a petri plate to climb on to the lid, although they did not leave a fluid one. They are attracted towards a source of heat or of light and are activated by both. Using the Goodey (4) technique of the skin of a young mouse stretched on a cork ring and floated on saline solution at blood temperature, the larvae were shown to penetrate the skin. This was confirmed by the percutaneous infection of ferrets. Infection, however, in the experiments reported was generally by mouth, which resulted in heavier crops of worms.

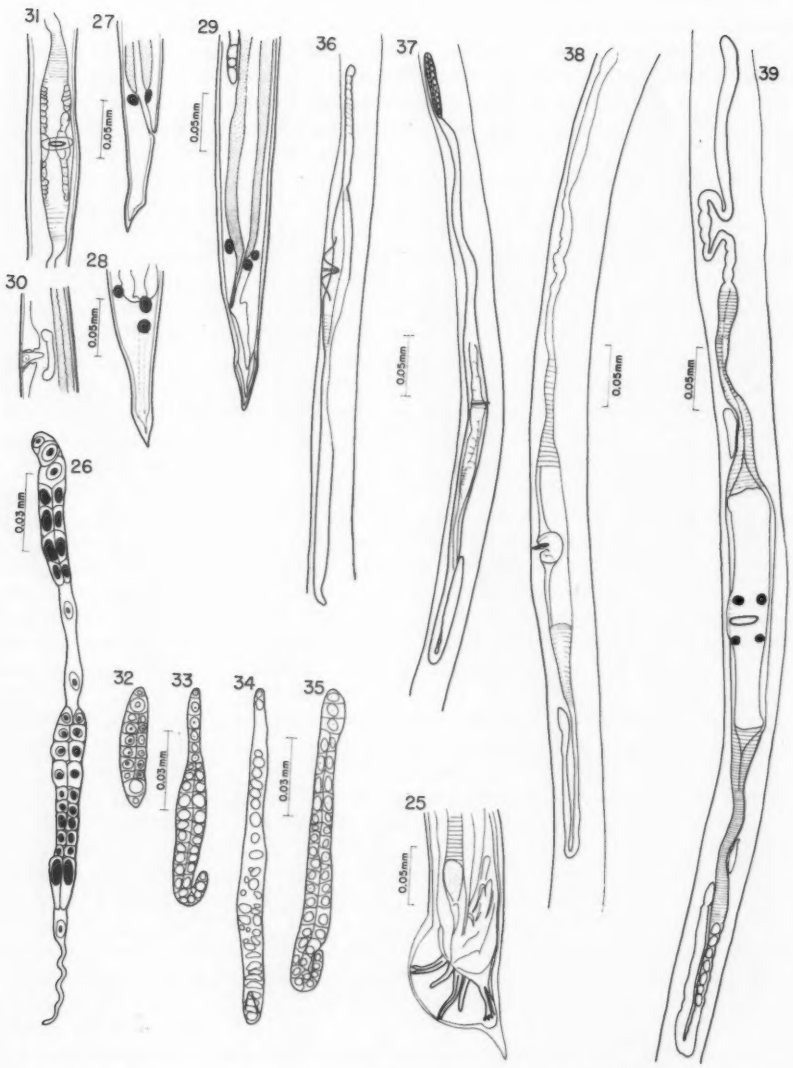
The third-stage larva exsheaths in the stomach of the ferret within 48 hours and lies close to the mucosa and apparently commences to feed at once. During the first 24 hours after infection there is an increase in the number of cells of the genital primordia and of the nuclei of the ganglia. As soon as the sheath is lost—during the second 24 hours in the stomach—there is an increase in length, as well as in number, of the genital and nerve cells, and the larvae leave the stomach and migrate to the small intestine. The lethargus is reached in about 72 hours after infection. The larva is now 1.58 mm long and sexual dimorphism is beginning to become apparent. In the *male* there is a slight thickening of the tail. The genital rudiment is increased in size and becomes differentiated and shows a development of the testis, the seminal vesicle, and the ejaculatory duct. It lies about 0.3 mm from the hind end and is almost 0.1 mm long.

In the *female* the tail is still pointed and the genital rudiment is not reversed but simply elongated anteriorly and posteriorly. It consists of a single genital cell at each end with a double row of 6–7 cells between them. It measures 0.02–0.05 mm in length by 0.01 mm in width and lies 0.14–0.25 mm from the hind end of the body.

During the lethargus the mouth apparatus, adapted to pierce the mucosa of the intestine of the host, is formed.



FIGS. 21–24. Fourth larval stage of *Molineus barbatus* third day after infection. Figs. 21, 22. Young male. Figs. 23, 24. Young female.



FIGS. 25–39. *Molineus barbatus*. Fig. 25. Development of male bursa. Fig. 26. Development of male gonad. Figs. 27–29. Development of tail of female. Figs. 30, 31. Development of vagina. Figs. 32–39. Progressive development of female gonads.

The Fourth-stage Larva (*Figs. 21–39*)

In the early fourth-stage larva, a provisional buccal capsule is present and the oesophagus is filariform. The excretory pore leads into an excretory sinus at the anterior end of the two cervical glands, each consisting of a single cell. No changes are seen in the nervous system.

In the *male* (Figs. 21 and 22), which is shorter than the female, the region round the anus is swollen, the swelling ending in a narrow, conical, slightly curved tail. This swelling continues until, by the time of the lethargus, the bursa and its rays have developed (Fig. 25). The genital primordium is greatly elongated and its three parts have become further differentiated (Fig. 26) and are recognizable.

In the *female* (Figs. 23 and 24), the tapering tail is bent dorsally (Figs. 27–29). The vulva is present, but the vagina is not yet fully developed (Figs. 30 and 31), although the genital primordium is greatly increased in size and is elongated or spindle-shaped, and differentiation is commencing. Its development is shown in Figs. 32–39.

The fourth lethargus takes place from the fifth to seventh day after infection but may be delayed as long as 13 days. It appears to be later in the females than in the males.

The Young Adult

The young adult (Fig. 40) has a small mouth cavity surrounded by 6 papillae but the cephalic inflation of the cuticle (Fig. 41), which is characteristic of the adult worm, is not yet developed. The filariform oesophagus (Fig. 42) is fully developed and the valvular flaps are seen projecting into the intestine. The excretory pore is conspicuous and the dorsolateral cervical glands are well developed.

In the *male* the genital primordium has grown backwards to unite with the rectum but, beyond an increase in size, its three portions are still much the same. The bursa is not completely developed, the lobes and rays being still indistinct. The spicules and gubernaculum are also incomplete and are still cellular masses.

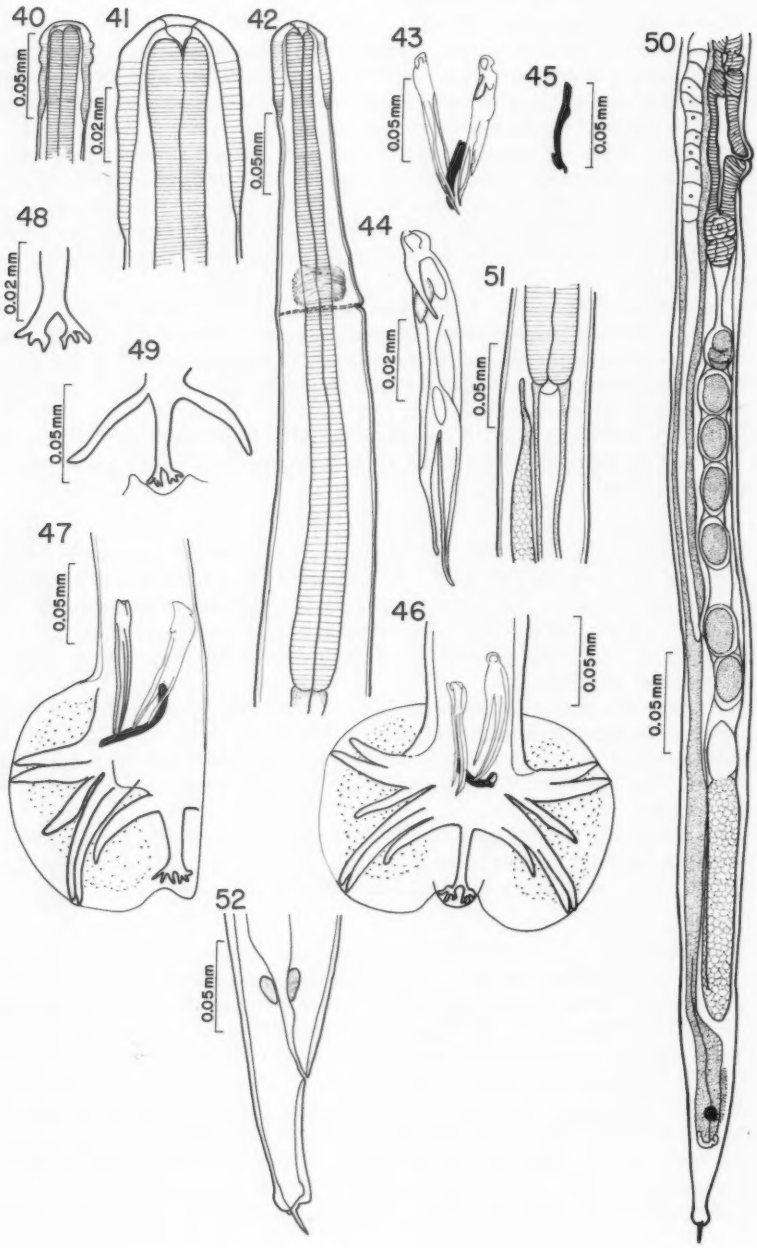
In the *female* the genital tube has become attached to the body wall and the primordium has extended at both ends. The vagina appears to be formed from cells in the body wall and unites subsequently with the ovejectors.

The Mature Adult

In the sexually mature adult, the characteristic cuticular inflation is present (Fig. 41). This has an anterior triangular mouth opening which communicates with the claviform oesophagus by a small tube; the mouth opening is surrounded by 4 papillae and 2 amphids. The swelling is transversely striated but no transverse striations are seen on the rest of the body, which, however, has 10–14 longitudinal striae. The excretory pore is conspicuous with two small lip-like swellings (Fig. 42). It is situated on the cervical groove and communicates with the two large, pear-shaped cervical glands.

The nerve ring is just anterior to the excretory pore while two small cervical papillae lie slightly anterior to the end of the oesophagus.

In the *male* (4.55–5.65 mm long), the testis is well developed and reaches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almost to the end of the oesophagus. The posterior end is united to the rectum. The spicules lie dorsal to the rectum and are equal and slender with an anterior 'barb' and two terminal pointed processes (Figs. 43 and 44). A small gubernaculum is present (Fig. 45).

The bursa is simple and continuous ventrally (Fig. 46). There are two large lateral lobes and a single small dorsal one: the inner surface of the lateral lobes is covered with minute bosses. The bursal rays are shown in Figs. 46–49 and do not differ from the original description by Chandler (3).

In the *female* (5.4–8.0 mm long), the gonads are well developed. The anterior ovarian tube is larger than the posterior and extends nearly to the oesophagus (Figs. 50 and 51); the posterior ovary is reflexed anterior to the anus and terminates just behind the vulva. When the female starts egg production about the 8th to 13th day after infection, the ovaries have increased in length and the uteri become distended with eggs. The vulva is postequatorial in position and the tail has developed its characteristic spine (Fig. 52).

While the males appeared fully mature in ferrets killed on the seventh day after infection, females contained no ova at this time and some were still in the fourth larval stage. Fully mature females were beginning to appear on the eighth day but most did not reach this stage until the 10th to 13th days.

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NOTE: Figure 53 follows.

FIGS. 40–52. Adult worms of *Molineus barbatus*. Fig. 40. Head of young adult. Fig. 41. Head of mature adult. Fig. 42. Anterior end of mature worm. Fig. 43, 44. Spicules of male. Fig. 45. Gubernaculum. Fig. 46. Dorsal view of bursa, spicules, and gubernaculum. Fig. 47. Lateral view of bursa, spicules, and gubernaculum. Fig. 48, 49. Details of dorsal rays. Fig. 50. Posterior end of mature female showing posterior gonad. Fig. 51. Anterior end of posterior gonad. Fig. 52. Tail of mature female.

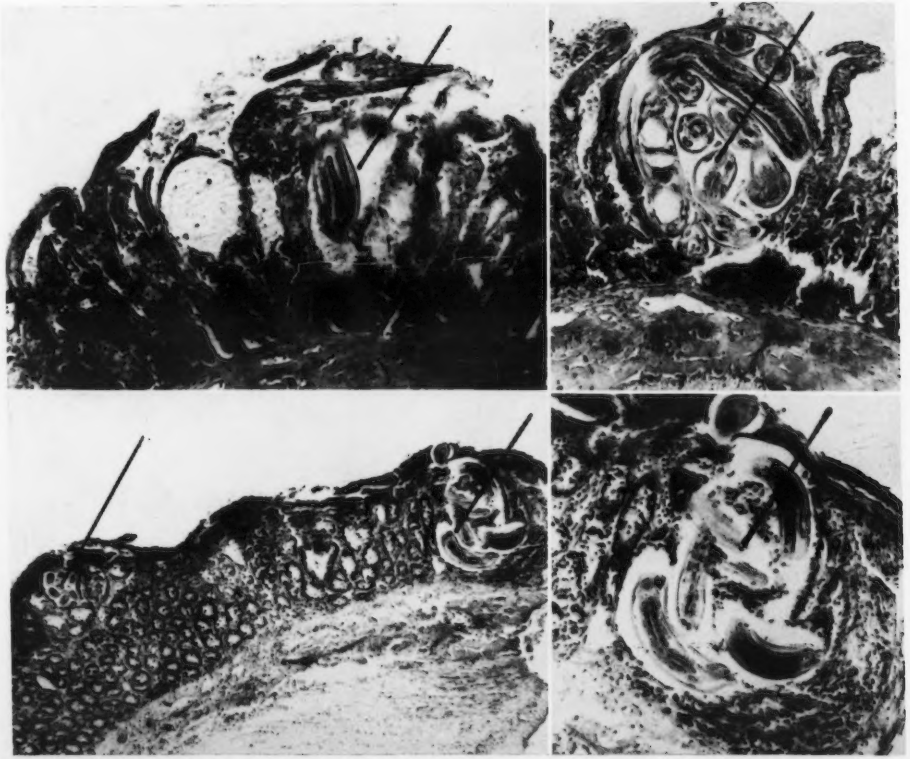

FIG. 53. Cross section of haemorrhagic intestine of a ferret, showing *Molineus barbatus* worms (indicated by lines).

Gupta—Can. J. Zool.

THE DISTRIBUTION AND IDENTIFICATION OF ESTERASES IN THE DEVELOPING EMBRYO AND YOUNG NYMPH OF THE LARGE MILKWEED BUG, ONCOPELTUS FASCIATUS (DALL.)[1]

E. H. SALKELD[2]

Abstract

Aromatic esterase (A esterase, organophosphate-resistant esterase), aliesterase (B esterase, organophosphate-sensitive esterase), and acetylcholinesterase were localized in the developing embryo and in the young nymph of the large milkweed bug, *Oncopeltus fasciatus* (Dall.). The esterase complex varied qualitatively with embryonic development. Aromatic esterase occurred in the 3-day-old embryo, aromatic esterase and acetylcholine-esterase were found in the 4-day-old embryo, while all three esterases occurred in the 5-day-old embryo and in the young nymph. The distribution of each esterase remained fairly constant during embryonic development; aromatic esterase was located in many tissues and cells, aliesterase occurred in the pericardial cells, and acetylcholinesterase was found only in the neuropile of the nerve cord and brain. In the young nymph, the number of sites of aromatic esterase activity was reduced while additional sites of aliesterase occurred; acetylcholinesterase activity remained in the neuropile.

Introduction

Cholinesterases and non-specific esterases occur in insect eggs. The cholinesterases have been associated with the nervous system and apparently function as an integral part of the cholinergic system as in adult insects (9). Non-specific esterases are present throughout the entire development of the egg but their physiological significance is not known. A few attempts have been made to characterize these esterases by their substrate specificity and their reactions to esterase inhibitors. Thus, esterases which hydrolyze *o*-nitrophenyl acetate and which are inhibited by TEPP-containing compounds (tetraethyl pyrophosphate) occur in very young eggs of *Diataraxia oleraceae* L. and *Ephestia kühniella* Zell. (7). An esterase hydrolyzing phenyl acetate occurs at all stages of development in eggs of *D. oleraceae* (8), *Pieris brassicae* L. (14), and in eggs of *Musca domestica* L. (4). The esterase demonstrated in the eggs of *M. domestica* was only slightly inhibited by 10^{-4} *M* eserine and by 10^{-7} *M* TEPP. It has recently been suggested that at least three esterases occur in the very young egg of *P. brassicae* while five distinct esterases are present in the almost fully developed egg (16). Only part of this total esterase activity was inhibited by 10^{-4} *M* paraoxon and by 10^{-4} *M* eserine.

Considerable confusion exists in the literature concerning the nomenclature of the various esterases found in insects. The interchangeable use of the terms aliesterase and non-specific esterase in some of the earlier work has added to this confusion. More recently (11), esterases have been classified according to their differential inhibition by carbamates and organophosphorus compounds

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[2]New York State Agricultural Experiment Station, Cornell University, Geneva.

into two groups: cholinesterases and non-specific esterases. The cholinesterases are broken down into acetylcholinesterase and pseudo-cholinesterase, while the non-specific esterases are divided into aromatic esterases and aliesterases among others. However, in the application of this classification method to insects many different carbamates and organophosphorus compounds at many different concentrations have been used, making the problem of correlating results difficult.

With few exceptions (15) most esterase determinations in the insect embryo have involved the use of tissue-brei preparations with their lack of precision in enzyme localization. Knowledge of the histological distribution of an esterase is essential for interpreting its physiological function. The present histochemical study was done to localize and identify the esterases that occur during the development of the embryo of the large milkweed bug, *Oncopeltus fasciatus* (Dall.). Embryonic development involves metabolic and differentiation processes not common to hatched forms and it is likely that the type and distribution of esterases in these two distinct developmental stages differ. Therefore, the histochemical study reported here also includes esterase determinations in the newly hatched nymph of *O. fasciatus*.

Methods

Eggs were removed from a laboratory colony of milkweed bugs by allowing the insects to oviposit through the wire-screen bottom of the cage onto a sheet of paper. Eggs collected over a period of about 8 hours were held at 26° C until 3, 4, or 5 days old; younger eggs were not used since very little differentiation of the embryo had occurred in the 1- and 2-day-old egg. The incubation period at this temperature was approximately 5.5 days. Young nymphs were collected within 1 hour after hatching.

Eggs and nymphs were cut into several pieces and fixed in cold 4% formol in 1% $CaCl_2$ for 2–24 hours. The fixative was then removed and the pieces of tissue incubated in various substrates. After an appropriate incubation time either paraffin-embedded or frozen sections were prepared.

Substrates used were α-naphthyl acetate, β-naphthyl acetate, naphthol AS acetate, 5-bromoindoxyl acetate, and acetylthiocholine iodide. The histochemical methods used were those outlined by Pearse (13). Since 5-bromoindoxyl acetate appeared to give the clearest and most reliable esterase picture, it was the substrate used most extensively.

Esterases were characterized by their reaction to various inhibitors before and during the period of hydrolyses in the substrate medium. Concentration series of the following inhibitors were used: eserine sulphate from 10^{-4} *M* to 10^{-6} *M*; *iso*-OMPA (tetra-*iso*-propylpyrophosphoramide) from 10^{-3} *M* to 10^{-7} *M*; paraoxon from 10^{-3} *M* to 10^{-7} *M*; DFP (diisopropyl fluorophosphate) from 6×10^{-5} *M* to 6×10^{-7} *M*. These were made up just prior to use in 0.2 *M* tris(hydroxymethyl)aminomethane–HCl buffer at pH 7.9. Tissues incubated in the inhibitor–buffer solution for 1 hour at room temperature were transferred to the substrate medium containing the same concentration of inhibitor and were incubated for an additional hour at 37° C.

Esterases were identified as cholinesterases if they hydrolyzed acetylthiocholine iodide and were inhibited by eserine at a concentration of 10^{-5}

M as well as by paraoxon at a concentration of 10^{-6} M. The cholinesterases were further classified as either acetylcholinesterase or pseudo-cholinesterase by the ability of the former to hydrolyze acetylthiocholine iodide in the presence of 6×10^{-6} M DFP or 10^{-6} M *iso*-OMPA. Esterases not inhibited by 10^{-5} M eserine were considered to be non-specific esterases. These were further classified as either aromatic esterase (A esterase, organophosphate-resistant esterase) or aliesterase (B esterase, organophosphate-sensitive esterase) by the ability of the former to hydrolyze the substrates in the presence of paraoxon at a concentration of 10^{-5} M. Non-specific esterases inhibited by this concentration were considered to be aliesterase (11, 13).

Since the embryology of *O. fasciatus* has been described by Butt (2), only a brief description will be given of each stage concerned in the present work.

Results and Discussion

Three-day-old Embryo

At this age, the embryo is in the process of, or has just completed, blastokinesis. Fat body, muscle tissue, and various components of the viscera are being formed. The neuroblasts are actively dividing to form columns of daughter cells but the neuropile is not yet formed in the nerve cord or brain.

Non-specific esterase activity, as evidenced by its insensitivity to 10^{-5} M eserine, occurred in many of the yolk granules, in the developing pleuropodia, and in the cells of the serosa. This activity was also insensitive to 10^{-5} M paraoxon, suggesting that it was an aromatic esterase. Non-specific esterase has been noted in young eggs of *D. oleraceae*, *E. kühniella*, and *P. brassicae* but its histological distribution was not determined (7, 14). Carlson (3) also found non-specific esterase in the yolk of the developing grasshopper egg and suggested that part of this esterase activity was in the serosa. Since the serosa is an actively secreting tissue involved in the production of part of the epembryonic or subchorial membrane (1), its esterase complement is not surprising.

Cholinesterases were not present at this stage of embryonic development since no inhibition of esterase activity was caused by 10^{-5} M eserine or by 10^{-6} M paraoxon. Tissue-brei preparations of young eggs of many species of insects have also indicated the absence of cholinesterases at this early stage in embryonic development (20).

Four-day-old Embryo

The lateral body walls have grown upwards and joined dorsally to enclose the yolk except in the area of the dorsal organ where the serosa is being absorbed into the yolk. The mid-gut epithelium now forms a trough under the yolk but has not yet enclosed it dorsally. The ganglia are distinctly bilobed and a small neuropilar area is present in each and in the brain.

As in the 3-day-old embryo, non-specific esterase activity was localized in the pleuropodia, in some yolk granules, and in the remnant of the serosa (Fig. 1). In addition, non-specific esterase activity also occurred in the dermal cells of the body wall and in some blood cells. This activity was considered to be due to aromatic esterase since there was no apparent reduction in activity at these sites after incubation in 10^{-5} M paraoxon. It is likely that the esterase observed in the epidermal cells of the embryo is involved in the for-

mation of the embryonic cuticle which become apparent at this time. The formation of this cuticle has not been studied in *O. fasciatus* but two such cuticles are produced by the ectoderm of the hemipteran *Dysdercus cingulatus* (Fabr.) (17).

Cholinesterase activity occurred in the nervous tissue and was confined to the neuropile of the ganglia and brain. This esterase was identified as acetylcholinesterase since it was completely inhibited by 10^{-5} *M* eserine and 10^{-6} *M* paraoxon but was not apparently affected by 10^{-6} *M iso*-OMPA or by 6×10^{-6} *M* DFP. Although slightly higher concentrations of DFP inactivated the enzyme, concentrations of *iso*-OMPA as high as 10^{-3} *M* did not. It also hydrolyzed acetylthiocholine iodide in the presence of the selective pseudocholinesterase inhibitor *iso*-OMPA. Other histochemical studies have shown that a cholinesterase occurs in the neuropile of the ventral nerve cord in a 6- to 7-day-old embryo of *P. brassicae* (15), adult *Rhodnius prolixus* (Stal.) (23), adult *Periplaneta americana* (L.) (5, 24), and adult *M. domestica* (5). As in several other insects (23, 5) no pseudo-cholinesterase was found in the nervous tissue of *O. fasciatus*. Although some non-specific esterase activity has been reported in certain parts of the nervous system in *Rhodnius* (23) and in the cockroach *P. americana* (24), no such activity could be found there in the embryo of *O. fasciatus*.

Five-day-old Embryo

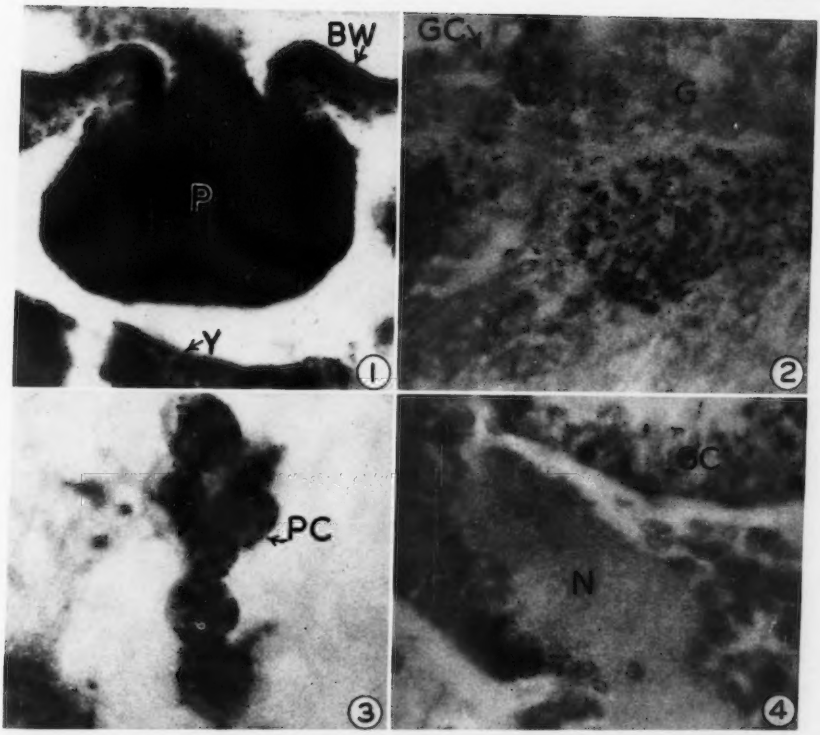
The embryo appears fully developed externally and is capable of movement. It is totally enclosed in an embryonic cuticle. All organs appear fully differentiated. The yolk is completely enclosed by the gut epithelium and has become a homogeneous mass. The nervous system has a very distinct neuropilar area in each ganglia and in the brain.

Non-specific esterase activity occurred in the gut cells (Fig. 2), in the contents of the gut, in the dermal cells of the body wall, the pleuropodia, many blood cells, pericardial cells (Fig. 3), Malpighian tubules, and in scattered oenocytes. Most of this activity, except that in the pericardial cells, is probably due to aromatic esterase since no apparent inhibition was caused by 10^{-5} *M* paraoxon. The activity in the pericardial cells is probably due to aliesterase since it was sensitive to this inhibitor. The esterase activity observed in the pleuropodia (see Fig. 1) in the 4- and 5-day-old embryo is undoubtedly involved in the partial dissolution of the epembryonic membrane which occurs at this time (1). The production of a "hatching" enzyme by the pleuropodia has been suggested for some other insects (10, 18) but the nature of the enzyme was not determined. The esterase activity in the epidermal cells is probably concerned with the production of the definitive cuticle of the embryo.

Acetylcholinesterase occurs in the neuropile of the nervous system (Fig. 2) as in the 4-day-old embryo. The complete inhibition of this enzyme by 10^{-5} *M* eserine while leaving the non-specific esterase in the gut cells unaffected is illustrated in Fig. 4.

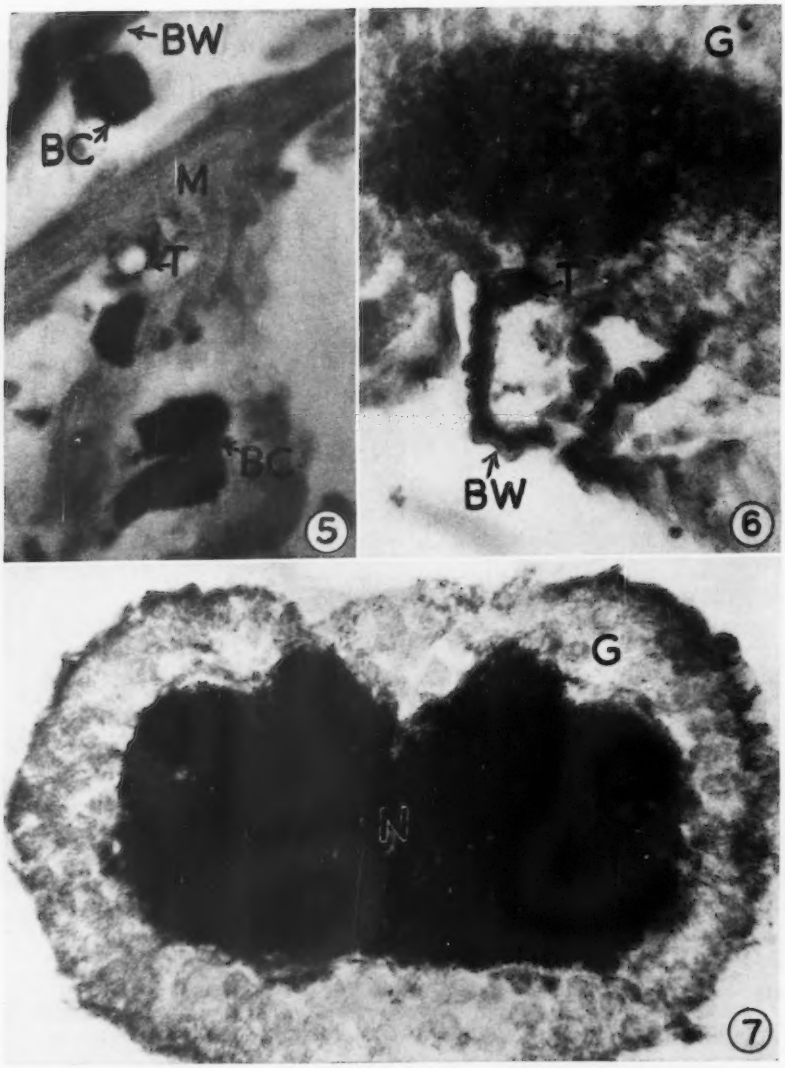
Young Nymph

Hatching occurs when the embryo splits the chorion and epembryonic membrane longitudinally by muscular force. The embryonic cuticle splits and remains within the egg-shell as the young nymph emerges. The nymphal



FIGS. 1–4. Sections of 4- and 5-day-old embryos of *O. fasciatus* showing localization of esterases by 5-bromoindoxyl acetate. Fig. 1, in pleuropodia, dermal cells, and yolk of the 4-day-old embryo, ×950. Fig. 2, in neuropile of ganglia and in gut cells of the 5-day-old embryo, ×570. Fig. 3, in pericardial cells, ×950. Fig. 4, in gut cells after incubation in 10^{-5} *M* eserine, ×570; note lack of esterase activity in neuropile of ganglion.

Abbreviations: BW, body wall; G, ganglion cells; GC, gut cells; N, neuropile; P, pleuropodia; PC, pericardial cells; Y, yolk.



FIGS. 5–7. Sections of the young nymph of *O. fasciatus* showing localization of esterases. Fig. 5, in blood cells, tracheae, and body wall by 5-bromoindoxyl acetate, ×475. Fig. 6, in neuropile of thoracic ganglion, tracheae, and some dermal cells of the body wall by α-naphthyl acetate, ×250. Fig. 7, in neuropile of subesophageal ganglion by α-naphthyl acetate, ×570.

Abbreviations: BC, blood cells; BW, body wall; G, ganglion cells; M, muscle; N, neuropile; T, tracheae.

cuticle is soft and pale orange in color but the head, thorax, and appendages harden and become black within about an hour after hatching.

The appearance of a non-specific esterase in the tracheal system occurs almost as soon as the chorion splits and allows free oxygen into the tracheae (Figs. 5 and 6); this esterase was not found in the embryo. Furthermore, it is probably an aliesterase since it is inhibited by 10^{-5} *M* paraoxon. Aliesterase occurred in the pericardial cells as it had in the embryo. Aromatic esterase similar to that observed in the dermal cells of the embryo is present in the epidermal cells of the head, thorax, and appendages until the cuticle of these areas darkens. Two rows of dermal glands extending from the anterior end of the thorax to almost the posterior end of the abdomen give a very conspicuous positive test for aromatic esterase at this time. These glands may be involved in the production of the outer layer of the epicuticle (22). Aromatic esterase was also localized in various other dermal glands, in the oenocytes, some blood cells (Fig. 5), gut cells, and Malpighian tubules. The pleuropodia have almost completely disappeared in the young nymph and no esterase activity remains there. No esterase activity was ever observed in the muscles (Fig. 5).

The ventral nerve cord has become shorter in relation to body length and the size of the ganglia has increased because of an increase in the neuropilar area rather than of the ganglion cell mass (6). Acetylcholinesterase, sensitive to 10^{-5} *M* eserine and to 10^{-6} *M* paraoxon, is abundant in the neuropilar areas of the nervous system (Figs. 6 and 7). Pseudo-cholinesterase and non-specific esterase were not observed in any part of the nervous system.

General Discussion

Aromatic esterase, aliesterase, and acetylcholinesterase have been localized histochemically in the developing embryo and in the young nymph of *O. fasciatus*. The esterase complement differed at each stage of development. For example, only aromatic esterase occurred in the 3-day-old embryo, aromatic esterase and acetylcholinesterase occurred in the 4-day-old embryo, while all three esterases were found in the 5-day-old embryo and in the young nymph. The sites of non-specific esterase activity remained fairly constant in the embryo while some striking changes occurred in the young nymph. The dermal cells which contained aromatic esterase in the embryo had lost much of this activity soon after hatching. Similarly the prominent aromatic esterase-positive pleuropodia of the embryo were no longer evident in the nymph. Aliesterase activity in the embyro was confined to the pericardial cells. Activity continued there in the nymph but also occurred in the tracheal system where no activity was observed in the embryo.

Acetylcholinesterase first occurred in the 4-day-old embryo coinciding with the appearance of the neuropile and was found only in the neuropilar area of the nervous system throughout development. This finding supports the conclusions drawn from egg-brei studies that the appearance of acetylcholinesterase in the embryo is closely associated with the development of the nervous system (14, 19). In the present work, esterases other than acetylcholinesterase could not be found in the nervous system. Wigglesworth (23) has recorded the presence of weak non-specific esterase activity in the ganglion

cells, the glial layer between these cells, and in the perineurium of the nervous system in adult *Rhodnius*. The lack of an esterase-positive reaction in these areas of the embryo and the young nymph of *O. fasciatus* likely reflects the true situation there since the differentiation of ganglion cells into morphological types is only partially completed at this time (6). However, it may also be the result of enzyme inhibition inherent in histochemical methods. Tissue-brei studies with isolated thoracic ganglia of adult *M. domestica* showed only 4% of the total thoracic non-specific esterase to be present in the ganglia (21); by analogy, any non-specific esterase that may occur in the ganglia of the embryo or young nymph of *O. fasciatus* is likely to be present in minute amounts.

As in *Rhodnius* (23), no sign of a positive esterase reaction occurred in any part of the muscles. This adds support to the evidence that neuromuscular transmission in insects is not cholinergic (12).

Acknowledgments

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EFFECTS OF REPEATED EXPOSURES OF HYPERSENSITIVE HUMANS AND LABORATORY RABBITS TO MOSQUITO ANTIGENS[1]

J. A. McKiel[2] and A. S. West[3]

Abstract

Two methods of altering reactivity to mosquito bites were studied, viz., repeated exposures of hypersensitive human volunteers to mosquito bites, and injections of bite-sensitive rabbits with mosquito extracts.

In one of the seven participating human volunteers, the delayed reaction was eliminated entirely while in three others certain aspects of this reaction were altered. Although pruritus was reduced in all subjects, no other feature of the immediate reaction became altered.

The level of hypersensitivity in rabbits remained unchanged following use of graded doses of mosquito extract as a desensitization procedure. However, a single intravenous injection completely desensitized rabbits temporarily. Extracts of *Aedes atropalpus*, *A. pionips*, and *Culex pipiens* were as effective in this procedure as was *A. aegypti* extract in desensitizing *aegypti*-sensitive animals.

Introduction

It is now well established that reactions to mosquito bites are allergic responses to the salivary fluids injected by the mosquito (9). These may take the form of immediate or delayed reactions or a combination of the two types. The immediate reaction appearing within minutes of the bite is the familiar wheal, commonly accompanied by erythema and pruritus, and typically is of short duration. The delayed reaction appearing some hours after the bite is a papular lesion, often associated with edema and intense, burning pruritus, and not infrequently persists for several hours or days. We have observed delayed reactions to bites of the yellow fever mosquito (*Aedes aegypti L.*) which caused discomfort for as long as 17 days.

The goal of our studies on reactions to insect bites has been to find a means whereby persons hypersensitive to these bites may be made refractory. It was evident that an approach to this goal would involve observation of the course of events when subjects, sensitized as a result of an unknown previous bite history, were exposed to a series of bites by one species of mosquito over a period of time. It was apparent also that subjection of laboratory rabbits to injections of mosquito extracts might provide information of value. It has been our experience that sensitization of laboratory animals to mosquito bites may be accomplished without difficulty (7, 9).

Studies by Mellanby (11) and others have shown that when bitten for the first time by mosquitoes an individual may exhibit no reaction whatever but

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[2]Formerly at Defence Research Kingston Laboratory; present address: Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Ontario.

[3]Department of Biology, Queen's University.

during a series of exposures over an extended period of time he may show the following stages of reactivity in sequence: (1) no reaction, (2) delayed reaction, (3) immediate and delayed reactions, (4) immediate reaction only, (5) a postulated final stage, no reaction.

The majority of persons who complain about the effects of mosquito bites are individuals who develop painful and persisting delayed reactions. In comparison, immediate reactions are relatively unimportant. Therefore a procedure which converts the type of bite reaction from stage 3 to stage 4 would be clinically beneficial even though stage 5 may not be reached. One frequently hears reports that Indians and Eskimos who live in heavily fly-infested areas in Northern Canada do not react to mosquito or black fly bites. While such reports require verification, their persistence at least suggests that complete desensitization is possible. A number of published reports suggest that some decrease in hypersensitivity may result from exposure to mosquito bites over a period of time.

Morse (12) stated that English and Irish immigrants suffered greatly from the effects of mosquito bites during the first year or so following their arrival in North America, but thereafter lost much of their sensitivity. According to Bowen (2), some individuals develop a seasonal immunity in about 10 days but react again, though less violently, the following year. Gordon and Crewe (5) reported the reactions of two subjects who received a series of mosquito bites during a 70-day period. One subject was bitten at regular intervals and became temporarily desensitized. The second subject was bitten at irregular intervals and failed to show any reduction in reaction. Gordon and Crewe's observations might suggest that regular exposures are essential for desensitization to occur, but the findings in only two subjects hardly justify a firm conclusion.

Saline extracts of mosquitoes have been used by several workers in attempts to reduce bite reactions in hypersensitive subjects. One of the patients treated by Benson (1) was not troubled by delayed reactions for four seasons after receiving a single injection of mosquito extract concentrated by precipitation with cold alcohol. Relief from delayed reactions only, and this of a very temporary nature, was reported by Brown *et al.* (3), Heilesen (6), and Rockwell and Johnson (13). One of the cases treated by Rockwell and Johnson developed severe edema and erythema on two occasions when more than a week elapsed between injections. Extracts of mosquito salivary glands did not reduce reactivity in subjects treated by McKinley (10).

The immediate reaction to mosquito bites in experimentally sensitized rabbits is likewise difficult to influence as shown by work reported by Dubin *et al.* (4), who used five rabbits previously sensitized to *Anopheles quadrimaculatus* (Say) bites. In an attempt to alter the reaction, they were given three injections consisting of 0.25, 0.5, and 1.0 ml of mosquito extract per week. For use in the first week's injections the extract was diluted 1:10,000, for the second week 1:1000, and for the third week 1:100. When tested for bite reactivity following this series, all rabbits but one reacted. A second series of injections of a crude suspension of *An. quadrimaculatus* failed to reduce the reaction further.

Methods

Mosquitoes used in the present study were either from laboratory-maintained colonies (*A. aegypti*, *A. atropalpus* (Coq.), and stenogamous, nonautogenous *Culex pipiens pipiens* (L.)), or were collected in the field as larvae and reared to the adult stage in the laboratory (*A. vexans* (Meig.)).

Extracts of mosquitoes were prepared by homogenizing freshly killed insects in buffered physiologic saline solution (pH 7), 10 ml of saline solution per gram of mosquitoes. The macerated mosquitoes were left in the saline solution at 6° C for 48 hours, following which extracts were filtered through paper, sterilized by Seitz filtration, and bottled aseptically.

The human subjects used in this study were volunteers composed chiefly of graduate students at Queen's University. They received their bites by placing the bared inner forearm against a cage containing mosquitoes. Immediate reactions were observed continuously during the ensuing hour and delayed reactions were recorded on the next and subsequent days for as long as they persisted.

Rabbits used were white males of a domestic mixed strain. They were sensitized to *A. aegypti* bites by five subcutaneous injections of 1 ml of *A. aegypti* extract given at daily intervals. Tests for sensitivity were made 10 to 14 days after the last injection by allowing two or more mosquitoes to feed on a depilated area of the skin.

The skin reaction of the hypersensitive rabbit consists principally of a wheal which begins to appear in 10 to 15 minutes after the bite and reaches its maximum elevation in about 1 hour. Following this it begins to flatten. Although the diameter of the wheal may continue to increase for 3 or more hours after the bite, the reverse sometimes occurs. Regardless of whether the diameter increases or decreases, as the wheal begins to flatten its borders become less distinct and consequently measuring the diameter becomes increasingly difficult. For this reason the standard procedure was to measure the diameter of the wheal 1 hour after the bite. This practice followed that adopted by Last and Loew (8), who found the 1-hour observation to be the most satisfactory for recording intradermal reactions of rabbits to a variety of agents.

Erythema is usually absent or if present its diameter is only slightly greater than that of the wheal. We have never seen any indication of a distinct and separate delayed reaction in hypersensitive rabbits.

Results

Two possible methods of altering the reactivity to mosquito bites were studied: repeated exposures to bites and injections of mosquito extracts.

Effect of Repeated Bites

This study was carried out in seven human volunteers who exhibited varying degrees of both immediate and delayed reactions to *A. aegypti* bites. Five of the seven were exposed to *A. aegypti* bites once-weekly in order to simulate week-end exposures out-of-doors while two subjects were exposed at irregular intervals. The total number of bites received by individual volun-

teers varied from 500 to 1500 and the periods of exposure from 3 to 12 months.

The principal features of the delayed reaction before and after these exposures are given for each subject in Table I. In three subjects (AB, GC, SB), the time elapsed between exposure and appearance of the delayed reaction (latent period) was shortened and in two of these (GC, SB), the duration of the delayed reaction was decreased. In subject LM, the delayed reaction was eliminated entirely during the 3-month exposure. The remaining subjects exhibited no change in these features of the reaction. By the time that the final exposure was made, all of the volunteers had reported that the pruritus associated with the immediate reaction had been markedly reduced. However, in no instance was there any change in the immediate skin reaction.

TABLE I

Effect of repeated exposure on the delayed reaction to *A. aegypti* bites in human volunteers

Subject	No. of bites	Exposure	Latent period of reaction, hr		Duration of reaction	
			Initial	Final	Initial	Final
AB	900	Wkly. for 7 mo.	18	1	1 day	1 day
DS	1500	Irreg. for 12 mo.	0	0	4 days	5 days
GC	1000	Wkly. for 5 mo.	4	1	1 day	5 hr
LM	1200	Irreg. for 3 mo.	0	—	3 days	—
PC	1100	Wkly. for 5 mo.	10	9	12 hr	12 hr
RS	500	Wkly. for 4 mo.	0	0	1 day	1 day
SB	1200	Wkly. for 5 mo.	9	4	4 days	1 day

Effect of Injections of Extract

Studies of the effects of injections of mosquito extracts on mosquito-bite reactivity were carried out in rabbits. Since rabbits develop only immediate reactions to mosquito bites, these studies consequently were restricted to immediate hypersensitivity.

A preliminary test was made in three *aegypti*-sensitive rabbits, using a course of injections patterned somewhat after that used by Dubin *et al.* (4). A mosquito extract was injected subcutaneously at daily intervals over a period of 35 days. The initial injection consisted of 0.1 ml of 1:10,000 extract. Successive doses were increased to a final injection of 1.8 ml of 1:100 extract. These rabbits were exposed to *A. aegypti* bites on the day following the last injection; one of the three rabbits failed to react. One week later the biting test was repeated and all three rabbits reacted as before the injection series.

In a second trial, 30 *aegypti*-sensitive rabbits were used. These were divided into two groups of 15 each. Both groups were composed of equal numbers of animals with strong and moderate wheal reactions. One group was injected subcutaneously with 1:10 extract and the other group was left untreated as a control. The course of injections lasted 49 days and was administered as follows: during the first 10 days, five injections each 1 ml; during the next 25 days, 18 injections each 1 ml; during the remaining 14 days, 10 injections each 2 ml. No observable adverse effects occurred following any of these injections.

All rabbits of the two groups were tested for reaction to *A. aegypti* bites

at the beginning and on the 11th, 35th, and 50th days of the experiment. In each of these tests except the last, all rabbits reacted to the bites. In the final test, one rabbit of the treated group failed to react. The results of the final biting test were analyzed statistically by means of the *t*-test and it was found that the mean wheal diameters of the two groups did not differ significantly at the 5% level of significance.

A third attempt to reduce the reaction of *aegypti*-sensitive rabbits was made using mosquito extracts injected by the intravenous route. Each of four rabbits was exposed to two *A. aegypti* bites. One hour later the bite reactions were recorded and each rabbit was injected intravenously with 2 ml of *A. aegypti* extract. Biting tests were performed 3 hours after making the injection and again on the 2nd, 3rd, and 4th days of the experiment. On the 6th day the injection was repeated and the skin reactions tested as previously. The results (Table II) show that all four rabbits were completely desensitized to *A. aegypti* bites 3 hours after the injections were made. Subsequent tests revealed, however, that the original sensitivity gradually returned in each rabbit.

The ability of *A. aegypti* extract to effect desensitization by the intravenous route was further tested in 13 rabbits. The results are included in Table III.

TABLE II

Effect of intravenous injections of *A. aegypti* extract on sensitivity of *aegypti*-sensitive rabbits

Day of experiment	Diameter of mosquito bite wheal (mm)			
	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4
1 (10 a.m.)	5.8	5.6	4.2	4.8
1 (11 a.m.)	Injected 2 ml *A. aegypti* extract intravenously			
1 (2 p.m.)	0	0	0	0
2	3.1	4.7	2.3	4.5
3	4.0	4.6	4.5	3.3
4	4.5	4.9	3.7	4.3
6 (10 a.m.)	5.4	5.4	4.7	4.6
6 (11 a.m.)	Injected 2 ml *A. aegypti* extract intravenously			
6 (2 p.m.)	0	0	0	0
7	0	4.2	2.0	4.3
8	3.3	5.2	3.1	4.8
9	4.0	5.7	3.8	4.9
13	6.4	5.8	4.8	5.6

TABLE III

Effect of an intravenous injection of mosquito extract on sensitivity of *aegypti*-sensitive rabbits

Extract injected	No. of rabbits injected	No. of rabbits shocked	Sensitivity* of rabbits to *A. aegypti* bites at given intervals after injection				
			3 hr	3 days	4 days	5 days	7 days
A. aegypti	13	1	12−	3−, 9+	3−, 9+	3−, 9+	12+
A. atropalpus	5	2	3−	1−, 2+	1−, 2+	3+	3+
A. pionips	3	0	3−	3+	3+	3+	3+
C. pipiens	5	0	5−	5+	5+	5+	5+
Saline solution	5	0	5+	5+	5+	5+	5+

*The figures which precede the + or − indicate the number of rabbits which reacted or failed to react, respectively, to *A. aegypti* bites.

One of the 13 rabbits died from anaphylactic shock. All of the 12 survivors were desensitized when tested 3 hours after injection. Three of these remained desensitized for 5 days but were sensitive again on the 7th day. These tests amply illustrate the transience of desensitization so far as the immediate reaction is concerned.

Specificity of Desensitization to Mosquito Bites

Gordon and Crewe (5) reported that a subject who became partially desensitized to *A. aegypti* bites continued to react as strongly as originally to bites of *C. molestus* Forskal suggesting that desensitization is highly specific. On the other hand, Rockwell and Johnson (13) reported that *A. aegypti* extract was found to be capable of desensitizing patients to the delayed effects of *C. pipiens* bites.

The observation that an intravenous injection of *A. aegypti* extract will desensitize *aegypti*-sensitive rabbits for at least 3 hours suggested a means of studying the specificity of desensitization. Extracts (1:10) of *A. atropalpus*, *A. pionips* Dyar, and *C. pipiens* were prepared and 2-ml amounts were injected intravenously into *aegypti*-sensitive rabbits. The numbers of rabbits injected and the results obtained are given in Table III. These data show that rabbits hypersensitive to *A. aegypti* bites may be desensitized temporarily not only by extracts of other *Aedes* species but also by an extract of a mosquito species belonging to a different genus (*Culex*) than the one used to initiate sensitization.

Summary

1. Seven human volunteers were exposed repeatedly for several months to *A. aegypti* bites. At the conclusion of the exposures, all subjects reported that itching caused by the bites was markedly decreased. The delayed reaction was altered in four subjects, but in no case was the immediate reaction significantly reduced.

2. Attempts to desensitize rabbits by means of graded doses of mosquito extract injected subcutaneously failed to effect a significant reduction of the immediate bite reaction. However, a single intravenous injection of *A. aegypti* extract rendered these animals temporarily nonreactive.

3. Extracts of *A. atropalpus*, *A. pionips*, and *C. pipiens* were found to be as effective in desensitizing *aegypti*-sensitive rabbits by the intravenous route as was *A. aegypti* extract.

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THE MORPHOLOGY AND HISTOLOGY OF THE ALIMENTARY CANAL OF GLOSSOGOBIUS GIURUS (HAM.)[1]

S. MOHD. MOHSIN

Abstract

Glossogobius giurus is a carnivorous fish with a wide mouth and a short alimentary canal. All the layers of tissue ordinarily found in the wall of the gut are well developed except in the buccal cavity and the pharynx where the muscular layers are missing.

The mucous membrane and the musculature vary greatly in the different regions. The muscularis mucosa is generally wanting. The stratum compactum is not well developed except in the buccal epithelium. There is a true stomach but no pyloric caeca. It is tentatively concluded that fish possess only pepsin-secreting gastric glands. Detailed descriptions are given of typical transverse sections of the various regions.

Introduction, Material, and Methods

The present work was undertaken with the aim of describing systematically and intensively the morphology and histology of the alimentary canals of various representative groups of Indian teleosts, and thereby of revealing whether any correlations exist between the habits, habitat, and food on the one hand and the nature of the digestive tracts on the other. Research on the anatomy, histology, and physiology of the alimentary tracts of fishes has been carried out by numerous workers in Europe, America, Asia, and elsewhere, but the investigators who have dealt with the digestive tracts of Indian fish are very few. Mention may be made of Dharmarajan (8), Vanajakshi (27), Sarbahi (24), Mohsin (19), and Ahsan-ul-Islam (1). Particular mention should be made of Rahimullah (21), who has done extensive work on the histology and physiology of the pyloric caeca. The Indian "gudgeon" or goby, *Glossogobius giurus*, a purely fresh-water fish which, according to Day, has a very wide distribution, being found in all parts of India, Burma, Ceylon, the Malay Archipelago, and even up to the Philippines, is the subject of the present paper. The author has also worked out similar details for 14 other species, his results being described in a thesis deposited in the library of the Osmania University.

The gudgeon were procured alive from tanks situated in the suburbs of Hyderabad, and the required material was obtained after pithing the fish. It was fixed in various fixatives but Bouin's fluid gave the best results. The time allowed for fixation varied from 12 to 24 hours. The fixed material was imbedded in paraffin and the sections cut 6–8 μ thick. A number of stains were used to verify and differentiate the cellular structure in the various regions, namely, Delafield's and Ehrlich's hematoxylin counterstained with eosin, Heidenhain's iron-hematoxylin, borax carmine counterstained with picro-indigo-carmine, and Mallory's triple.

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Contribution from Department of Zoology, Osmania University, Hyderabad-Deccan, India.

General Morphology of the Gut

Since the Indian gudgeon is a carnivorous fish, its gut is comparatively short (Fig. 1). The liver is huge and practically fills the whole abdominal cavity. The bile duct opens into the loop between the stomach and the intestine.

The buccal cavity is quite long and wide. The cleft of the mouth is wide and the lips not very thick. The tongue is notched and attached to the floor of the buccal cavity. The pharynx is small and has minute pharyngeal teeth, arranged on two small pads. The oesophagus is very small and tubular. The stomach is well developed; its cardiac region is somewhat swollen and the pyloric region is tubular and elongated. The pylorus is not very distinct but, on dissection, it can be located by the presence of a sphincter. The intestine is very small and has two loops. The rectum is distinct, being slightly greater in diameter than the intestine. A sphincter occurs instead of an intestinorectal valve.

Although the relative lengths of the various regions of the canal are subject to considerable variations, the measurements on a single fish, 15.8 cm long (excluding the caudal fin), given in Table I will give an idea of their order.

TABLE I

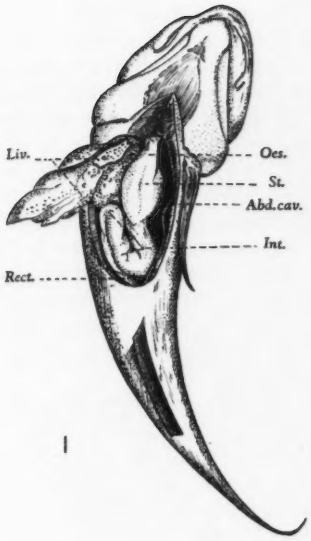
Organ	Length, cm	Width, cm
Buccal cavity and pharynx	2.2	0.9
Oesophagus	1.3	0.3
Stomach	1.9	0.6
Intestine	3.1	0.4
Rectum	1.2	0.5

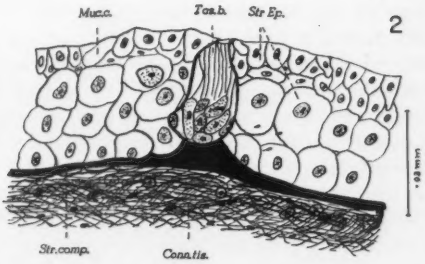
Histology

All the layers of tissue ordinarily found in the wall of the gut are well developed except in the pharynx and the buccal cavity. The mucous membrane and the musculature show great histological variations in the different regions. The muscularis mucosa is generally wanting. The stratum compactum is not well developed in the tract of this species except in the buccal epithelium where it is distinctly seen. Granular cells are discernible in certain regions.

The membrane lining the buccal cavity consists of stratified epithelium which rests on a basement membrane. Below it lies a layer of loose connective tissue fibers containing minute blood vessels. The epithelium is slightly corrugated.

The tongue (Fig. 2) consists of an epithelial region, made up of epithelial cells and taste buds, etc., and a subepithelial region composed of areolar connective tissue. The epithelial region of one particular specimen was 30 μ thick. The epithelial region is composed of four or five tiers of stratified epithelial cells. The cells in the first two or three rows are flattened. Below these, the cells assume a cuboidal form with rounded nuclei. Still lower in the epithelium the cells are much larger and are arranged with their long axes





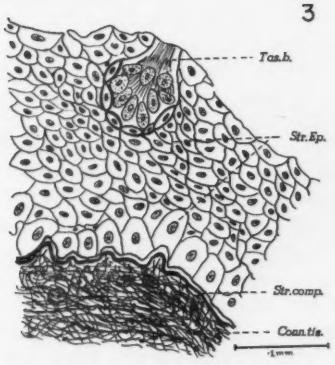


FIG. 1. (×2) Dissection of *Glossogobius giuris* from the ventral aspect, showing the disposition of the alimentary canal and the large liver *in situ*. For explanation of abbreviations used in figures see list below.

FIG. 2. Camera lucida sketch of the tongue, transverse section, showing a taste bud and very prominent stratum compactum.

FIG. 3. Camera lucida sketch of the floor of the buccal cavity, transverse section, showing a taste bud and the disposition of the stratum compactum.

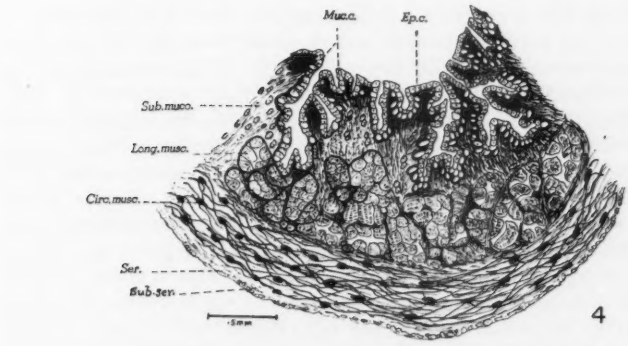
ABBREVIATIONS USED IN FIGURES

Abd. cav.	Abdominal cavity.
Bl. cap.	Blood capillaries.
Circ. musc.	Layer of circular muscle-fibers.
Conn. tis.	Connective tissue.
Ep. c.	Epithelial cells.
Gast. Ep.	Gastric epithelium.
Gast. fl.	Folds of the gastric mucosa.
Gast. gl.	Gastric glands.
Gob. c.	Goblet cells.
I. l.	Intermediate (myenteric) layer.
Int.	Intestine.
Int. vil.	'Intestinal villi'.
Leuc.	Leucocytes.
Liv.	Liver.
Long. musc.	Layer of longitudinal muscle-fibers.
Lum.	Lumen.
Muc. c.	Mucous (or goblet) cells.
Oes.	Oesophagus.
Rect.	Rectum.
Ser.	Serosa.
St.	Stomach.
Str. comp.	Stratum compactum.
Str. Ep.	Stratified epithelium.
Sub. muco.	Submucosa.
Sub. muco., E.	Extension of the submucosa into the muscle layers.
Sub. ser.	Subserosa.
Tas. b.	Taste bud.
Tun. prop.	Tunica propria.

more or less at right angles to the surface. The nuclei of these lower cells are also rounded and their nucleoplasm is reticular in appearance. A deeply stained nucleolus is visible in each nucleus. Goblet cells, with elongate nuclei towards their bases, are usually present near the surface of the epithelium. Taste buds are few in number and are situated on papillae. Each taste bud is enclosed within a definite sheath lined by flattened cells which are quite prominent towards the basal part of the bud. The nuclei of these basal cells are elongated. The gustatory cells are more or less oval with elongate nuclei. The upper processes of a group of eight to ten of these cells fuse together as a band and give the appearance of hairlets as is found in mammals. Each taste bud measures about $18 \times 12\,\mu$. The subepithelial region is composed mostly of areolar connective tissue in which large numbers of granular cells are found. This region is fairly thick, being about 16–20 times the epithelial region. Just below the epithelial region is a thick band of stratum compactum which provides a sort of support to the epithelial layer and also forms a base for the taste buds. In this layer, as well as in the connective tissue matrix, fine blood vessels are discernible.

As in the case of the tongue, the floor of the buccal cavity (Fig. 3) is also divided into two regions. The epithelial region is stratified as in the tongue. The cells towards the upper surface are in transverse rows and are more or less flattened with rounded nuclei. Those below this layer are somewhat cuboidal. Still lower they gradually assume columnar form with their long axes at right angles to the surface. Taste buds are found towards the upper surface of the epithelial region, with gustatory cells like those in the tongue. The epithelium of the floor of the buccal cavity is thrown into low folds. The subepithelial region of the floor of the mouth consists of a dense network of areolar connective tissue fibers. Intermingled are some elastic fibers and large numbers of granular cells. Just below the epithelial region a thick band of stratum compactum extends towards the papillae of the epithelial layers but does not reach the bases of the taste buds.

The oesophagus (Fig. 4) shows a very much folded condition of the mucosa, the folds being quite long and pleated, hence leaving but a small lumen between them. The mucosa consists of two or three layers of small, more or less oval, epithelial cells, full of mucin and having their nuclei towards their bases. Simple mucous glands are also seen just below or adjacent to the mucous cells, amongst which some undifferentiated cubical cells are present. The latter have rounded nuclei, mostly situated towards the center of each cell. The submucosa is well developed and compact, forming a more or less solid core to the mucosal folds. Numerous granular cells, fine blood vessels, and nerve fibers are enclosed within it. The layer of longitudinal muscle fibers is represented by a large number of muscle bundles distributed in the submucosa and, as in other fishes, its position relative to the circular muscle is reversed from that which ordinarily obtains. These muscles are not in continuous layers but are disposed, more or less alternately, in two groups one lying below the other. Some of the fasciculi penetrate deep, even into the submucosal core of the mucous folds. The layer of circular muscle is very thick and consists of comparatively short, loosely arranged fibers with spindle-shaped nuclei.



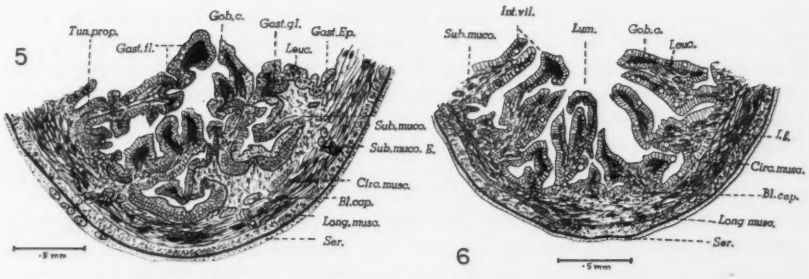


FIG. 4. Camera lucida sketch of the oesophagus, transverse section, showing very prominent mucous (or goblet) cells and the nature of the longitudinal layer of muscle bundles in definite fasciculi.

FIG. 5. Camera lucida sketch of the stomach, transverse section, showing very simple type of gastric glands.

FIG. 6. Camera lucida sketch of the intestine, transverse section, showing the much-branched "intestinal villi".

The whole of the interspace between the muscle fibers is filled up with areolar connective tissue. The subserosa is well marked off. It consists of areolar connective tissue amongst which blood vessels and nerves are located. The serosa is thin and consists of a single layer of epithelial cells.

The stomach (Fig. 5) has the mucosa highly folded and branched, presenting a beautiful arborescent condition which fills practically the whole of the gastric lumen. In some places the folds fuse together forming small lacunae. The mucosa consists of two or three layers of columnar epithelial cells with rounded nuclei situated towards their bases. Goblet cells are few and situated well within the mucous epithelium. Leucocytes also occur in the mucosa in fairly large numbers. The gastric region as a whole is not well defined. The gastric glands are of a simple type consisting of aggregations of small gland cells. The cells are somewhat rounded with their nuclei mostly towards their centers. Each nucleus has a deeply-staining nucleolus. The cell plasma is full of zymogen granules. All the gland cells are grouped as a mass around a very small tubule or duct. There are but few gastric glands. The submucosa is well developed and compact. It is highly vascularized and innervated. The

tunica propria is seen but a stratum compactum and a stratum granulosum are not discernible. The layer of circular muscle consists of thickly set fibers in which many spindle-shaped nuclei are visible. At places the submucosal connective tissue strands penetrate deeply across this muscle layer. The layer of longitudinal muscle is about one-third the thickness of the circular layer. The intermediate or myenteric layer is not very distinct. The subserosa is fairly well developed, vascularized, and innervated. The serosa is thin and consists of a single layer of epithelial cells.

The intestine (Fig. 6) also has a highly folded and branched mucosa with anastomoses at places so that lacunae are formed in the sections. One of these is discernible in the figure next to the intestinal villi. The mucosa consists of at least two rows of columnar epithelial cells whose nuclei are rounded and generally confined to the basal half of the cell. Goblet cells occur in large numbers and are found along all parts of the intestine. Leucocytes are also seen in the upper third of the mucous membrane in moderate numbers. A top plate is present on the surface of the mucosa. The submucosa is well developed. It consists of thick strands of connective tissue fibers enclosing a large number of granular cells, capillaries, and nerve fibers. The musculature of the intestine does not show any peculiarity and consists of a thick layer of circular muscle fibers and a thin layer of longitudinal ones. The intermediate or myenteric layer is also visible. The serosa and subserosa are not well defined although present.

The small intestine directly joins with the large intestine or rectum, and externally there does not seem to be any marked difference between these two regions. The histological features of the rectum are as follows. The mucosa of the rectum is similar to that of the intestine, but it is much more folded and the columnar epithelium consists of a number of layers of cells. The nuclei with nucleoli are scattered in the epithelial cells. Goblet cells are abundant. Leucocytes are present among the columnar cells of the epithelium. The submucosa is very much reduced. The musculature of this region consists of a layer of circular muscle and another of longitudinal as usual. The intermediate layer is not clear. The serosa and subserosa form a single epithelial layer.

Discussion

The Indian gudgeon possesses a true stomach but lacks the pyloric (intestinal) caeca which occur in so many other fishes. But it is certain that the presence of pyloric caeca bears no definite relation to the diet of the fish, for they sometimes occur in all three types of fishes, namely, carnivorous, herbivorous, and omnivorous, and sometimes, as in the present case, are absent altogether.

Some workers have not distinguished between the pharynx and the oesophagus. Although there are no external indications there exist certain histological differences between the two regions. I agree with Dawes (7) in differentiating them since three features distinguish the pharynx from the oesophagus: (i) the absence of longitudinal muscle, (ii) the presence of a definitely stratified epithelium with its abundance of goblet cells, and (iii) the presence of taste buds. Since Dawes, the occurrence of taste buds in the pharynx of fish has now

also been described by Rogick (23), Imhof (13), Curry (6), Sarbahi (24), Mohsin (19), Al-Hussaini (2, 3, 4), Ahsan-ul-Islam (1), and Kapoor (16, 17).

There are further histological differences which distinguish the pharynx from the oesophagus. The mucosal folds are generally much more branched in the oesophagus. The stratified epithelium of the pharynx generally disappears and columnar epithelium takes its place. The mucosal folds are abundantly beset with goblet cells. The main observation made by the present author, in agreement with others, is that the arrangement of the muscle layers is reversed in the oesophageal region as compared with the other succeeding parts of the alimentary canal in that the circular layer of muscle fibers is external. Al-Hussaini (3) mentions the occurrence of only one layer of muscle fibers, i.e. circular, in the oesophagus of *Mulloides auriflamma*, while all other workers have recorded two layers in the musculature. It may be that the minutely dispersed bundles of the longitudinal muscle fibers escaped his observation.

The present author has not been able to discover any taste buds in the oesophagus of *Glossogobius*, although MacCallum (18) has mentioned their presence in the oesophageal mucosa of *Acipenser*. The oesophagus of *Glossogobius* lacks a ciliated epithelium, although its presence has been described by Sullivan (25), Purser (20), and Mohsin (in manuscript) in *Barbus* (*Puntius*) *sarana*.

The development of mucous glands, I believe, is correlated with the feeding habits of the fish and the nature of the food, and I agree with Dawes (7) in asserting the main functions of the pharynx and the oesophagus to be gustatory and mucus-producing, and that the secretion of the mucous glands is concerned with the easy swallowing of food.

An interesting aspect of the gastric glands is their presence or absence in the two regions of the stomach, the cardiac and the pyloric. Greene (11) states that the gastric glands do not occur near the pyloric valve. Ishida (15) asserts that no gastric glands are found in the pyloric region of *Mugil cephalus* and that the same is true of the cardiac region. Al-Hussaini (3) records their absence in the pyloric region of *Mulloides auriflamma*. So far as the observations of the present author, as well as those of other investigators, are concerned, it may be said that it is the cardiac stomach where gastric glands are abundant (Dawes (7), Ghazzawi (10), Vanajakshi (27), Mohsin (19), Al-Hussaini (3)). While it may be said that in general the cardiac region is the site of most gastric glands it has also been observed that the pyloric region of certain fishes is studded with some regular, simple, and definitely tubular gastric glands. Dawes (7) remarks that "near the pyloric sphincter the gastric glands become shallow", indicating their presence, and Berndt (5) has described multicellular glands near the pyloric orifice in *Anguilla fluviatilis*.

With regard to the cells of the gastric glands, it is a common observation (e.g. Edinger (9), Gulland (12)) that in fish they are not differentiated into parietal or oxyntic and central or peptic cells. This is further supported by Rahimullah and Das (22). They have demonstrated by biochemical tests and physiological experiments that the stomachs of *Ophicephalus striatus* (carnivorous) and of *Osphronemus goramy* (herbivorous) secrete pepsin only. Hence it may be tentatively said that fishes probably possess one kind of cells only

in their gastric glands and these are pepsin-secreting. The question of the source of the acid was solved by Stirling (25), who showed the possibility of the superficial cells performing a dual function, that is they may produce acid as well as mucus.

The intestinal epithelium possesses a typical structure consisting of columnar and mucous (goblet) cells. The mucous folds simulate villi and crypts. Intestinal glands, analogous to the lacteals and the glands of Lieberkuhn, are not found. Leucocytes occur in moderate numbers between the cells of the epithelium. It may be mentioned here that the intestinal epithelium of *Glossogobius* is devoid of cilia although their occurrence in this region has been mentioned by Ishida (14) and Mohsin (in manuscript) in *Pseudorhombus triocellatus* and *Lagocephalus lunaris*.

There exists a controversy among the various authors with regard to the differences found between the histological structure of the intestine and rectum. The present author, in view of the facts studied, is of the opinion that the rectum in general, and that of *Glossogobius* in particular, differs from the intestine in (i) the greater degree of mucosal folding, (ii) considerable thickening in the musculature of the rectal wall, and (iii) the greater abundance of the mucous cells. Dawes (7) mentions an intestinorectal valve in the plaice, and Al-Hussaini (2, 3, 4) an ileorectal valve in the fishes investigated by him. However, such a valve has not been observed either in *Glossogobius*, *Anabas testudineus*, or any other Indian fish studied by the present author. As a substitute for the valve, a distinct sphincter is found at the junction of the two regions. Sarbahi (24) has described certain conical cells in the rectum of *Labeo rohita*. Such cells have not been observed by any of the previous or more recent workers, including the present author. The rectum of *Glossogobius* does not bear any cilia, as have been recorded by the present author in some other teleosts, such as *Nandus nandus*, *Sillago sihama*, and *Centrogenys waigiensis* (in manuscript).

Acknowledgments

I wish to express my great indebtedness to the late Professor B. K. Das for suggesting this problem to me and for all his constant help, friendly criticism, guidance, and encouragement. My best thanks are due to the Director of the Biological Survey of India for the loan of some rare and valuable literature references. I am also grateful to Mr. Ibraim Ali, former photo-artist of the Zoology Department, Osmania University, for his assistance in the preparation of the diagrams. I have great pleasure in thanking Professor F. E. J. Fry for his editorial help.

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REDESCRIPTION OF THE DIGENETIC TREMATODES LEPIDAPEDON CALLI AND L. PUGETENSIS ACENA, AND NEW HOST RECORDS FOR L. CALLI AND EURYCREADIUM VITELLOSUM MANTER FROM FISHES OF WASHINGTON STATE[1]

HILDA LEI CHING

Abstract

Lepidapedon calli Acena, 1947 and *L. pugetensis* Acena, 1947 are redescribed from types and paratypes and from additional specimens of *L. calli* collected at Friday Harbor, Washington, U.S.A. *L. pugetensis* is synonymized with *L. elongatum* (Lebour, 1908) Nicoll, 1915. The pleuronectid fish *Microstomus pacificus* is a newly reported host for *L. calli* and for *Eurycreadium vitellosum* Manter, 1934. *Steringotrema* (*Rhodotrema*) *quinquelobata* Layman, 1930 is transferred to the genus *Eurycreadium*, becoming *E. quinquelobata* (Layman, 1930), because of its tubelike excretory bladder, extensive vitellaria, and type of cirrus sac.

Introduction

The trematodes found in *Microstomus pacificus*, a pleuronectid fish commonly called the Dover sole, were collected at the Friday Harbor laboratories in Washington State, preserved in acetic acid – formalin alcohol, stained with Semichon's carmine, and mounted in permount. Types and paratypes of *Lepidapedon calli* Acena, 1947 and *L. pugetensis* Acena, 1947 were borrowed from John S. Rankin, Jr. and are now on deposit in the Helminthological Collection of the U.S. National Museum.

Hanson (2) questioned the placement of *L. calli* in the genus *Lepidapedon* and considered *L. pugetensis* a *species inquerendum* on the basis of insufficient description. In many instances, the figures drawn by Acena (1) conflicted with his descriptions. Studies of his types and paratypes have resolved this conflict. Following are the redescriptions of the two species as well as a report of a new host record for *Eurycreadium vitellosum* Manter, 1934.

All measurements in the following are in millimeters except where noted; drawings were made with the aid of a camera lucida.

Family Lepocreadiidae

1. *Lepidapedon calli* Acena, 1947 (Figs. 1–3)

Hosts.—*Parophrys vetulus* (type host).
Microstomus pacificus Pleuronectidae (new host record).

Location.—Digestive tract of *P. vetulus*, intestine of *M. pacificus*.

Locality.—Eliot Bay (according to label on slide of Acena's paratype), and Friday Harbor, Washington.

Frequency.—Large numbers in both hosts.

Redescription

Remeasurements of Acena's specimens and 15 additional specimens from

[1]Manuscript received May 23, 1961.
Contribution from the Department of Zoology, University of British Columbia, Vancouver, B.C.

TABLE I

Measurements of *Lepidapedon calli*

	Acena (1947) range	Measurements by Ching of Acena's: Holotype	Measurements by Ching of Acena's: Paratype	Range and average of 15 specimens from *M. pacificus*
Length	0.94–1.17	1.237	1.211	0.969–1.246 (1.070)
Width	0.35–0.40	0.365	0.343	0.325–0.422 (0.379)
Forebody		0.376	0.291	0.325–0.427 (0.367)
Oral sucker	0.11 by 0.09	0.142 by 0.154	0.103 by 0.114	0.117–0.149 (0.132)
Ventral sucker	0.11–0.12 by 0.095	0.131 by 0.142	0.114	0.095–0.123 (0.109)
Genital pore	Dextrad	Sinistral	Sinistral	Sinistral
Prepharynx	0.02–0.06 by 0.02	0.034	0.028	0.01–0.071 (0.036)
Pharynx	0.06 by 0.095	0.085	0.063 by 0.085	0.073–0.097 by 0.055–0.071 (0.082 by 0.062)
Oesophagus	0.035 by 0.02	0.063	0.040	0.026–0.086 (0.059)
Anterior testis	0.121–0.125	0.160	0.148	0.084–0.117 by 0.123–0.169 (0.102 by 0.146)
Posterior testis	0.12–0.13 by 0.15–0.16	0.171	0.142	0.091–0.130 by 0.124–0.187 (0.118 by 0.147)
Cirrus sac: length	0.16	0.285	0.239	0.119–0.313 (0.258)
width 1		0.068	0.058	0.058–0.078 (0.064)
width 2	0.10–0.13	0.149	0.142	0.094–0.156 (0.113)
Ovary	0.11–0.13	0.142	0.131	0.081–0.123 by 0.095–0.130 (0.097 by 0.110)
Eggs	80–89 by 40 μ	78–81 by 32–42 μ	78–91 by 38–45 μ	61–84 by 30–45 μ

M. pacificus show the species to have a greater range in size than indicated by Acena (Table I). Sizes and proportions of the various organs in Acena's specimens compare favorably with my collections. Acena drew the figures and described the genital pore and ovary from a dorsal view but had not indicated this. Furthermore, the structure and shape of the cirrus sac differ from his description. Vasa efferentia from the testes join to form a wide, common duct which enters the cirrus sac (Fig. 2). The cirrus sac is bipartite, with a narrow isthmus lacking gland cells; the anterior part contains the cirrus and straight inconspicuous seminal vesicle. The posterior part, containing the coiled seminal vesicle and very abundant prostatic cells, is what has been referred to as the "external seminal vesicle" of *Lepidapedon* species. The anterior part of the cirrus sac in my specimens extends 0.019–0.039 (0.033) anterior to the ventral sucker and is 0.058–0.078 (0.064) wide. The posterior part has a width of 0.094–0.0156 (0.113). Because Acena did not describe the anterior portion of the cirrus sac, he confused those who looked for a bipartite sac as is found in other species of *Lepidapedon.*

Description of Two Immature Forms (Fig. 3)

Body length, 0.456, 0.598; width, 0.131, 0.177. Oral sucker diameters, 0.073, 0.091; ventral sucker, 0.058, 0.066. Forebody approximately 39 per cent of body length, 0.182, 0.233. Prepharynx shorter than pharynx, 0.016, 0.032. Pharynx round, 0.034, 0.039. Oesophagus almost the same length as prepharynx, 0.022, 0.039. Ovary, 0.039, 0.045 in diameter. Testes slightly larger than ovary: anterior testis, 0.055, 0.071; posterior testis, 0.058, 0.074.

In comparison with adults, the immature specimens are more elongate but with similar spination (anterior half of body spined), oral to ventral sucker ratios, and extent, but not development of vitellaria. Prepharynx and oesophagus are more equal in length, pharynx round and weakly muscular. Cirrus sac gourd-shaped with numerous gland cells and inconspicuous seminal vesicle.

Comparisons

This species resembles *L. lebouri* Manter, 1934 and *L. rachion* (Cobbold, 1858) Stafford, 1904 in the extent of the vitellaria and size-ratios of oral and ventral suckers, but *L. lebouri* is very large and elongate with vast post-testicular area and *L. rachion* has a very short oesophagus and oblong pharynx. The form of the cirrus sac is similar to that of *L. congeri* Manter, 1954 but the anterior seminal vesicle of *L. calli* is inconspicuous, the oral sucker larger than the ventral sucker, and the excretory bladder and caeca shorter.

2. *Lepidapedon elongatum* (Lebour, 1908) Nicoll, 1914 (Fig. 4)

Synonym: *Lepidapedon pugetensis* Acena, 1947.

Host.—*Sebastodes nebulosus.*

Location.—Intestine.

Locality.—Puget Sound, Washington.

Redescription

Measurements of *L. pugetensis* as given by Acena and measurements of the holotype and paratype made by me are presented in Table II. The holotype and paratype are much smaller in size than Acena has indicated in his paper.

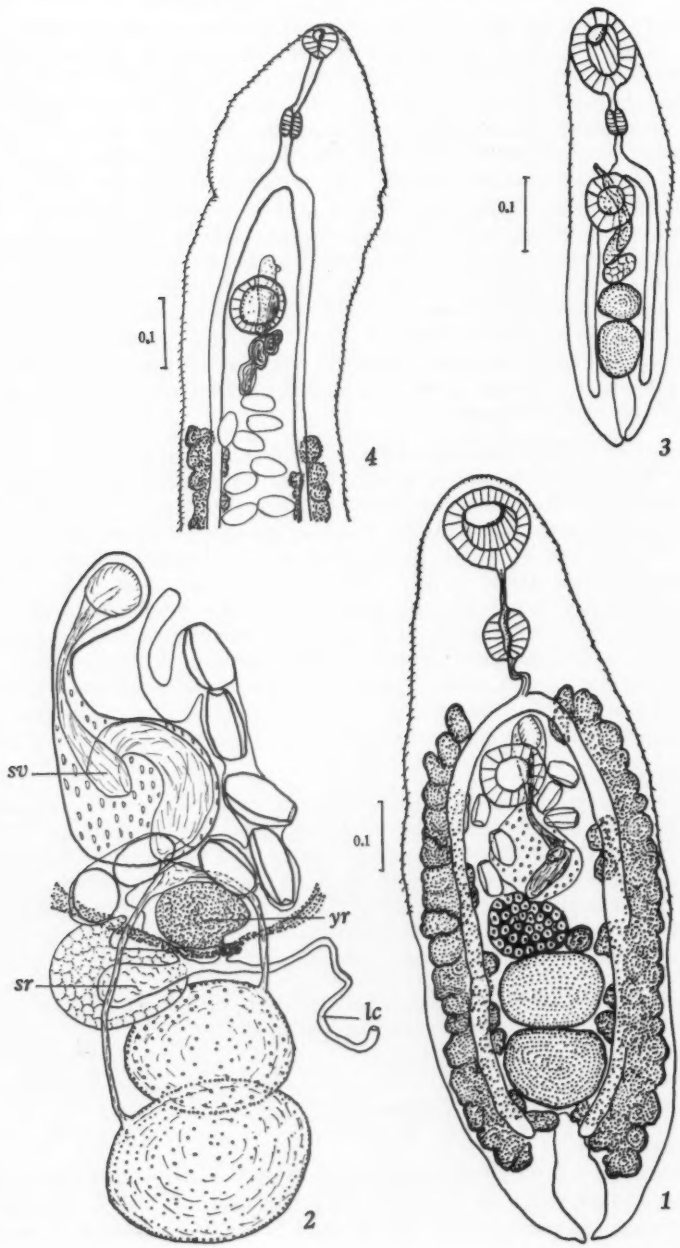
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TABLE II

Remeasurement of *Lepidapedon elongatum* (=*L. pugetensis*)

	Acena range	Measurements by Ching of Acena's: Holotype	Paratype
Length	2.37–2.47	1.852	1.653
Width	0.32–0.37	0.343	0.245
Oral sucker	0.04–0.05	0.032 by 0.040	0.052
Ventral sucker	0.10 by 0.09	0.071 by 0.065	0.092
Forebody	1.97	0.343	0.370
Prepharynx	0.12 by 0.03	0.045	0.078
Pharynx	0.06 by 0.045	0.045 by 0.026	0.045 by 0.029
Oesophagus	0.06 by 0.01	0.029	0.053
Anterior testis	0.07 by 0.14	0.117	0.117 by 0.130
Posterior testis	0.19 by 0.15	0.136	0.110 by 0.182
Ovary	0.15 by 0.12	0.097 by 0.120	0.123 by 0.103
Eggs	90 by 45 μ	69–75 by 29–34 μ	(for both specimens)

The anterior end of the body is heavily spined; spines begin to lessen at the level of the gonads and are completely absent at the posterior end instead of being evenly distributed as in Acena's figure. Sucker ratios of the two specimens were 1:1.6 and 1:1.8; the ventral sucker is not at least twice the size of the oral sucker. There is a definite prepharynx which is equal to or 1½ times longer than the pharynx. The pharynx is not especially large in relation to the rest of the body. Eggs measuring 90 by 45 μ were not found in the type specimens.

The inadequately described cirrus sac is bipartite. The anterior portion is elongate and contains the inconspicuous seminal vesicle, cirrus, and scanty prostatic cells. The irregularly shaped posterior portion has a highly coiled seminal vesicle and few prostatic cells.

The elongate body, distinct oesophagus and prepharynx, distribution of vitellaria, placement of ventral sucker well posterior to the intestinal bifurcation, and the wide intertesticular space are characteristic of *L. elongatum*. Because of these similarities, *L. pugetensis* is considered a synonym of that species.

Family Opecoelidae

3. *Eurycreadium vitellosum* Manter, 1934

Host.—*Microstomus pacificus* (new host record).

Location.—Intestine.

Frequency.—Three specimens in one of 14 hosts.

Locality.—Friday Harbor, Washington.

Numbers on scales represent millimeters.

FIGS. 1–3. *Lepidapedon calli* from *Microstomus pacificus*. Fig. 1. Ventral view. Fig. 2. Semidiagrammatic drawing of reproductive organs, dorsal view: lc, Laurer's canal; sr, seminal receptacle; sv, seminal vesicle in posterior portion of cirrus sac; yr, yolk reservoir. Fig. 3. Young specimen, ventral view.

FIG. 4. Anterior portion of *Lepidapedon elongatum* as drawn from Acena's paratype, ventral view.

Description

Body smooth, length, 1 to 1.3; width at level of ventral sucker, 0.431–0.634. Oral sucker subterminal, round, 0.125–0.150 in diameter; prepharynx lacking; pharynx, 0.053–0.088 by 0.058–0.070. Oesophagus shorter than pharynx, 0.035–0.049. Ventral sucker with longitudinal aperture, 0.255–0.295 in diameter, nearly twice the diameter of oral sucker. Genital pore opening opposite the middle of the pharynx. Cirrus sac club-shaped, extending slightly posterior and dorsal to ventral sucker in two specimens, to the right and not reaching past the mid-ventral sucker in the other specimen. Seminal vesicle undivided, prostate gland weakly developed, cirrus long and narrow. Testes smooth, large, slightly oblique to tandem, oval to round. Ovary with three to four lobes, lateral to right caecum, slightly overlapping the caecum dorsally at the posterior level of ventral sucker. Oviduct long and coiled. Uterus pretesticular with few coils extending anterodorsally to ventral sucker. Seminal receptacle within uterus. Laurer's canal coiled posterior to ovary, opening dorsally. Vitellaria mostly dorsal, abundant throughout body from level of pharynx to middle of testes and overlapping organs within the area. Eggs measure 64–75 by 29–35 μ. Excretory vesicle diamond-shaped, overlapping anterior edge of testes, surrounded by gland cells before terminating at excretory pore; excretory pore terminal.

Discussion

The specimens differ from the original description in the shorter oesophagus and pharynx, tapered instead of enlarged ends of the caeca, and extent of the vitellaria to the mid-testes instead of posterior to the testes. The ovary appears to be mostly lateral to the right caecum with part of the lobes overlapping dorsally. The eggs are slightly smaller, 64–75 by 29–35 μ instead of 82–84 by 36–40 μ.

Manter (3) transferred two fellodistomatids, *Rhodotrema skrjabini* Issaitchikow, 1928 and *R. problematicum* Issaitchikow, 1928, to *Eurycreadium* because of their extensive vitellaria, sac-shaped excretory vesicle, and type of cirrus sac. Yamaguti (6) also transferred a species, *Rhodotrema lethrini* Yamaguti, 1939, to this genus in support of Manter's belief that the worms only superficially resembled fellodistomatids. However, Skrjabin and Koval(5) retain these three species in the original genus of fellodistomatids and also included *Rhodotrema quinquelobata* Layman, 1930 and *R. quadrilobata* Baskikalowa, 1932 (which Shulman and Shulman-Albova (4) considered synonymous). The latter species, which is regarded by Yamaguti (7) as *Steringotrema* (*Rhodotrema*) *quinquelobata*, is now transferred to *Eurycreadium*, becoming *E. quinquelobatum* (Layman, 1930). *E. quinquelobatum* is similar to *E. vitellosum* but appears to differ in the location of the genital pore and ovary and the extent of vitellaria.

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ACTIVITY OF TWO SPECIES OF CALLIPHORA (DIPTERA) DURING BAROMETRIC PRESSURE CHANGES OF NATURAL MAGNITUDE[1]

D. K. EDWARDS[2]

Abstract

The amount of flight activity of *Calliphora vicina* R.D. was monitored during (*a*) natural, diurnal, barometric pressure changes, and (*b*) experimental pressure changes of natural amounts and rates. Activity was increased by a change in trend from rising or level to falling pressure, or by two step-like drops of 1 millibar, each lasting 15 minutes, separated by 1 hour of level pressure. The *change* to falling pressure, not falling pressure *per se*, stimulated activity in *C. vicina*. Activity was little, if at all, affected by a change to rising pressure from a previous condition of falling or level pressure.

With *Calliphora vomitoria* (L.) no effects of a changing pressure trend on activity were observed.

These findings indicate that natural, and particularly prefrontal, pressure drops under certain conditions can influence the amount of activity of some insect species.

Introduction

Sixty-three years ago, Sajo (13) reported that insects in the field became more active during periods of barometric depression. Since then, such pre-storm activity in insects has been widely observed, but very little studied. Cook (2) made some comparisons between weather and the number of moths obtained in traps, but he concluded that there was less correlation between pressure and the number trapped than between temperature or humidity and numbers. However, Cook made only a daily record of pressure, and there was no indication of the pressure trend during any one day. In 1931, Uvarov (15) stated that, "On the whole we must conclude that the influence of atmospheric pressure on the activities of insects remains at present practically unstudied". There have been some later observations of the activity of insects in the field in relation to weather. Underhill (14) observed that certain simuliids fed more readily during rapid falls of pressure, or during low pressures. Lewis (reported by Wellington (19)) obtained more insects in traps during prefrontal falls in pressure than during postfrontal rises, irrespective of temperature changes. Henson (11) reported an association of mass movements of spruce budworm and passages of cold fronts. Kettle (12) observed different degrees of abundance of biting flies with different kinds of weather conditions, but he made no mention of barometric pressure.

A summary of the major reports of pressure effects on insects up to 1946 has been given by Wellington (17). Also, there have been some reports of effects of pressure on biological functions in insects other than activity, but the present report is concerned only with the latter. Very few experiments

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Contribution No. 759, Forest Entomology and Pathology Branch, Department of Forestry, Ottawa, Canada.
[2]Forest Entomology Laboratory, 409 Federal Bldg., Victoria, B.C.

have been done to try to isolate the effects of pressure on activity from effects of other environmental parameters also associated with approaching storms (e.g., atmospheric electricity, temperature, humidity, and light changes). Furthermore, the author has been unable to find any reports of effects of small and gradual pressure changes on insect activity. In most experiments with pressure reported, either the pressures used, or the rates of change from one level to another, have exceeded the corresponding limits found in nature at a constant altitude. Some studies on pressure effects on insect activity have been reported by Wellington (17) and Haufe (10). Wellington (16) also reported some experiments in which the pressures used were those found at several, considerably different altitudes. This latter work was intended to study the effects of altitude, and considerable variations in pressure were simulated; the rates of change of pressure were also rapid, imitating in effect the transport of insects in vertical air currents to high altitudes.

The purpose of the present paper is to report the results of some experiments on blowfly activity when barometric pressure varies within natural, and sometimes very small, limits at a constant altitude near the ground, with other environmental factors relatively constant. Two species were studied, *Calliphora vicina* R. D. and *Calliphora vomitoria* (L.). The activity of groups of insects was continuously recorded (*a*) during natural barometric pressure changes indoors, and (*b*) during artificial pressure changes in a pressure chamber.

Activity Method

The two species of insects used in these studies were reared from eggs on a specially prepared culture medium (8). The stock adults were maintained indoors in wooden cages exposed to artificial light for 15 hours each day.

Some of the activity responses to small pressure changes were expected to be fairly transitory. Consequently, a method permitting only intermittent estimates of the activity changes could not be used. A continuous record of the amount of flying activity in a group of insects was obtained with an electrical technique, a detailed description of which has already been published (5). Briefly, the principle is as follows. When *Calliphora* (or, for that matter, any flying insect) takes off from one point within an earthed, metal, cage and flies to another part of the cage, the insect acquires an electrical potential at take-off. A probe fixed in the center of the cage is connected externally to an electrometer (i.e. a d-c. amplifier with an ultra-high input resistance which is highly sensitive to static electric charges; the Keithley Model 600 electrometer used here will register about 2×10^{-4} volts). With each flight within the cage there is a sudden deflection and return to "zero" of the electrometer indicator. This is recorded as a sudden deflection from a "base line" drawn by a strip-chart pen-recorder (Varian G-10) connected to the output of the electrometer. By counting the number of deflections per unit time which exceed an arbitrary length, an activity curve can be obtained by plotting the number of deflections against time. For convenience, only the deflections on the positive side of the base line were counted; the ratio of deflections on either side of the base line in these experiments was constant,

the negative deflections usually being "rebound" effects. The strip-chart was printed with eight divisions per hour, giving a basic time unit of 7.5 minutes per chart division at the chart speed used.

Natural Pressure Changes

Method

Approximately 100 *C. vicina* adults were kept in the laboratory in a cage measuring 7.6×5.1×5.1 decimeters. The frame was of wood, but two sides were covered with wire screen and two with wood. The top was glass, covered with wire screen. One wooden end, which faced out into the room, was covered by a piece of sheet aluminum. All metallic coverings were connected to earth, and the entire cage rested upon an earthed wire screen. This amount of electrostatic shielding was necessary to nullify the fields from external static charges which otherwise would influence the probe within the cage (e.g., movements of persons in the vicinity). In addition, the cage was shielded from direct natural light, and a 60-watt light bulb provided 15 hours of illumination per day.

The electrometer voltage range was set at 0–0.03 volts; the resistance between probe and earth was 10^8 ohms; the chart speed was 2 in. per hour, each chart division representing 7.5 minutes. The activity record was begun at 1000^h and terminated at 1600^h P.D.T., during 9 days, from July 19–29, 1960. Simultaneous records of pressure in the room were obtained with a microbarograph, and records of room temperature and humidity were also made. The activity record of the flies for each 6-hour recording period was later compared with the corresponding pressure record.

The activity record of the 100 *C. vicina* was compared with the barograph record of natural changes in pressure on nine different days. The activity record was divided into two parts. The average number of deflections per chart division (7.5 minutes) for the period 1000^h to 1300^h was subtracted from the average number for the period 1300^h to 1600^h. Thus, if the difference was positive, the afternoon activity exceeded that in the morning; if the difference was close to zero, there was little or no difference in activity between the afternoon and morning periods. No evidence was seen, in studies with artificial pressure changes (below), of an inherent diurnal increase in activity in *C. vicina* in the afternoon, compared with earlier in the day. (Backlund and Ekeroot (1) also found no endogenous daily cycle of activity in this insect. The activity is primarily dependent upon light intensity (1, 3).) Prior to 1300^h there were three different pressure conditions: level pressure, rising pressure, and falling pressure. In all the records obtained, the pressure following 1300^h trended downward. By comparing the difference in activity between afternoon and morning with the corresponding difference in barometric pressure trend, it was possible to determine a relationship between change in the pressure trend and change in the amount of activity.

Table I shows the average activity values (deflections per chart division), morning and afternoon, on the nine days of the records, together with the differences between the morning and afternoon values. Also indicated are the pressure trends during the morning and afternoon periods, and the cor-

TABLE I

Average activity values (deflections/chart div.) for two time-periods, with differences, together with the natural barometric pressure trend during the two periods, for *C. vicina*

Date, July	1000h–1300h Mean deflections	1000h–1300h Barometer trend	1300h–1600h Mean deflections	1300h–1600h Barometer trend	p.m. minus a.m.	Temperature (°C) a.m.	Temperature (°C) p.m.
20	4.095		6.592		2.497	24	26
22	3.809		6.291		2.482	21.5	24
27	2.791		5.500		2.709	24	28
19	3.791		4.791		1.000	24.5	27
21	3.130		4.920		1.790	23	24.5
25	2.541		3.291		0.750	24	27
26	4.208		3.083		−1.125	26.5	27
28	3.666		4.333		0.667	24.5	26
29	2.916		3.708		0.792	25	28

responding room temperatures. There is a relationship between the amount of change in pressure trend from morning to afternoon, and the difference in activity between morning and afternoon. With a change from rising pressure in the morning to falling pressure in the afternoon, there was a definite increase in the afternoon activity over that of the morning. With a change in trend from level in the morning to falling pressure in the afternoon, there was a moderate increase in the afternoon activity. When the pressure was dropping during both morning and afternoon, so that there was *little or no change in pressure trend*, there was very little increase in afternoon activity (in one case a decrease).

The temperatures indicated in the table show that a slight amount of warming occurred both on days when there was no change in pressure trend, and on days when there was a change in trend. This warming did not mask the activity differences occurring as a result of different pressure conditions, although it was probably responsible for the very slight increase in activity on the days when there was no change in pressure trend.

The absence of a significant change in activity between mornings and afternoons with no change in pressure trend was later supported by some observations made during the experiments with 12 *C. vicina* in the pressure chamber. On Aug. 23, when the barometer was falling naturally all during the day, the value for the difference *afternoon activity minus morning activity* was −0.041. On Aug. 25, the barometer remained absolutely steady and level throughout the day; the activity difference in this case was −0.062. In both of these later experiments, temperature, humidity, and illumination did not vary.

Artificial Pressure Changes

Method

(*a*) *Apparatus*

Experiments were carried out in which the activity of insects in a pressure chamber was monitored while the pressure was varied by natural amounts and rates. Close control of air pressure in the chamber was facilitated by the development of two special pieces of apparatus. One was a bleeder valve (4) which made possible accurate pressure adjustments as small as 0.1 millibar; the other was a programming device, through which it was possible to preset the rate of long, steady pressure-drops required. A brief description of the programmer follows.

A horizontal turntable, 36 cm in diameter, was attached to the drive-shaft of a 24-hour clock mechanism. Near the turntable was a slide-valve which delivered to the pressure chamber positive air-pressure, zero pressure, and negative pressure, depending upon the position of the valve. A lever-arm was attached to the slide-valve, so that horizontal movements of the lever-arm changed the valve position. A pointed stylus was fixed to the distal end of the lever-arm, and this rested upon the surface of the turntable. The lever was pulled toward the central pivot of the turntable by an elastic band. It was possible to program the position of the air-valve by fastening a specially cut template, or simply a French Curve, to the surface of the turntable and resting the end of the lever-arm against the curved edge of the template. With the turntable slowly rotating (1 rev. in 24 hours) the lever-arm followed the contour of the template, thus slowly changing the setting of the air-valve to the pressure chamber. The air pressure within the system was monitored by a kerosene manometer, and by a 24-hour microbarograph sealed in a glass case connected to the pressure chamber.

The greatest difficulty in these experiments resulted from unpredictable, external, barometric pressure changes, because it was not possible to obtain a commercial barostat capable of counteracting completely all types of natural changes (i.e., providing control to ± 0.5 mb). Sometimes during the experiment, the external barometric pressure changed in such a way as to neutralize, or reverse, the experimental pressure trend which was programmed. Some experiments had to be discontinued as a result of this. Most experiments, therefore, had to be carried out on days when the barometer was fairly steady, or at least when the trend in the barometer was predictable. It was often necessary, therefore, to obtain a rough prediction of the probable pressure trend on the morning prior to an experimental run, from the local Dominion Meteorological Observatory.

The entire apparatus was located in a darkroom. The insects were illuminated for 15 hours each day with a 60-watt tungsten bulb through the glass door of the pressure chamber. The temperature in the darkroom was $23 \pm 1°$ C; R. H. $47.5 \pm 2.5\%$.

(*b*) *Procedure*

Experiments were performed with *C. vicina* to study the effects on activity of four different pressure profiles: (*a*) a period of fairly steady, level, pressure followed by a period of steadily dropping pressure (approximately 1 mb per

hour); (*b*) a period of fairly steady pressure followed by two step-like drops (1 mb in 15 minutes) separated by 1 hour of steady pressure, and with steady pressure at the third new level following the second drop; this resulted in a step-like pressure profile; (*c*) a step-like pressure profile similar to (*b*), but with rising pressure instead of falling pressure; and (*d*) a period of steadily rising pressure immediately following a period of steadily falling pressure.

Experiments (*a*) and (*b*) were repeated at a later date using *C. vomitoria*. The rate of pressure drop in these steadily falling pressure experiments ranged from 1 to 5 mb per hour.

Each group of insects used consisted of six males and six females. The insects were put into a cubical tin box with sides of 23 cm, two opposite sides of which were fitted with wire window-screen (5). The electrometer probe projected through the top of the box to a point in the center. Within the box was a supply of water and sucrose crystals for food. The box was put into the pressure chamber, and the probe connected to a highly insulated cable leading externally to the electrometer.

Experiments were not begun until the day following that in which the insects were put into the apparatus. The same group of insects was usually used on several successive days, since they lived for many days relatively undisturbed in good condition in the apparatus. They were disturbed only when the pressure chamber door was unsealed at the end of the day to ensure adequate ventilation during the night, and when the door was sealed again in the morning. When the pressure chamber was sealed during the experiments, there was a slight air-flow through the system, but not enough to influence the insects mechanically.

For the activity records, the electrometer settings were 0–0.01 volts, and 10^{10} ohms. The experimental changes in pressure did not affect the electrometer probe. The zero setting of the electrometer, and hence the position of the "base line" drawn on the recorder chart, could be shifted only if the pressure was rapidly changed by several millibars; even then, this produced a "d-c. shift", and it would not have affected the interpretation of the deflections occurring *perpendicular* to the base line on the chart.

Results

(*a*) *Steadily-falling Pressure*

The activity curve and barometric pressure for an entire experiment with *C. vicina*, on Aug. 25, are shown in Fig. 1. The activity curve was obtained from the chart record in the manner outlined above. The figure shows two important phenomena: (*a*) the long period during the day (1100^h–1600^h) of practically no pressure change was accompanied by no general change in the amount of flying activity; (*b*) the period of artificially falling pressure (1600^h–1900^h) resulted in a definite increase in activity. During the entire experiment, temperature, humidity, and light were constant.

Figure 2 compares the effect on activity of a change from level pressure to steadily falling pressure, in *C. vicina* and *C. vomitoria*. Each point in Fig. 2 represents the mean of three points from three experiments. An explanation of the method used in analyzing the data follows.

In all these experiments the initial period of 2 hours prior to the beginning of the pressure drop was taken as the control. First, all the data from the

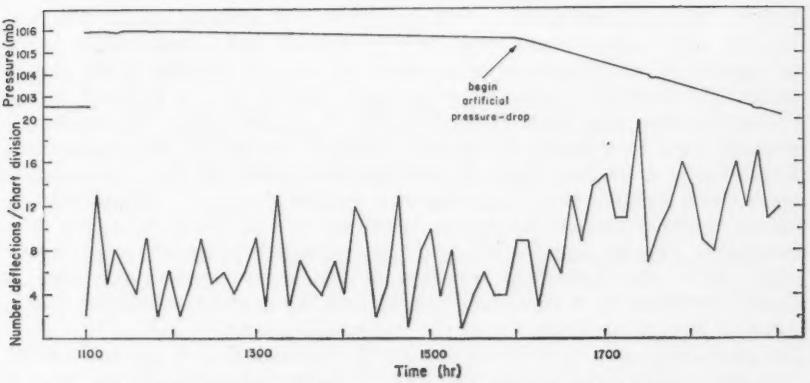

FIG. 1. Activity (number of deflections per chart division) of *C. vicina* in one experiment prior to, and during, a change in pressure trend from level pressure to steadily falling pressure.

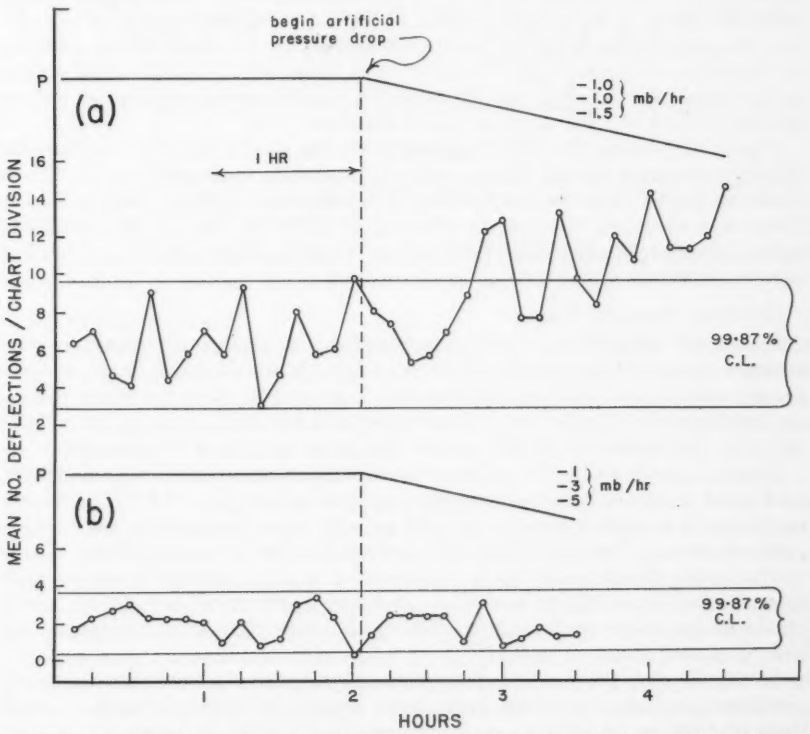

FIG. 2. Activity, from three experiments, during a change from level to steadily falling pressure (*P*): (*a*) *C. vicina*, (*b*) *C. vomitoria*. 99.87% confidence limits were calculated for initial period of level pressure.

control period for one species were corrected to a common mean. The data from the three experiments could then be pooled, and a mean value ($n=3$) for number of deflections was calculated for each chart division in the control period. The method for describing confidence limits about a series of data in a time sequence was taken from Goulden (7, pp. 418–426). This method is normally used in industry in "quality control" checks for the maintenance of quality in a uniform flow of manufactured products. Any deviation of *means*, from samples in a time sequence, outside the pair of calculated confidence limits indicates that some condition has occurred to cause these samples to deviate significantly from the accepted limits of normal variability. All of the individual standard deviations are pooled, the result is slightly modified by a correction factor, and the confidence limits are then obtained by taking three times the corrected, pooled, standard deviation. The probability of a mean lying outside the limits is 0.13% (from 3×S.D.).

In the present experiments, the 99.87% limits calculated on the basis of the initial control period of pooled data were extended across the experimental portion of the pooled curve. Thus, during the experimental period, any *persistent* deviation of means from the limits established for the control period could be taken as highly significant. It should be emphasized at this point that the significant deviations were considered to be those which *persisted* as deviations in the time series, rather than those which were isolated points. An isolated mean outside the limits in the control period suggests an experimental artifact at that point in one of the runs.

Figure 2(*a*) shows that the change from steady, level pressure to a condition of falling pressure (about 1 mb per hour) gradually increased the activity of *C. vicina*. Curve (*b*) shows the activity of *C. vomitoria* under similar conditions. There was obviously no activity response to a change from level pressure to falling pressure (1, 3, and 5 mb per hour in three experiments), within the time limit of the experiment.

(*b*) *Short Pressure Drops*

On several different days when the barometer was relatively steady, experiments were carried out to determine the effects of well-defined, short drops in pressure on the activity of *C. vicina* and *C. vomitoria*. After a control period, an initial drop of 1 mb (over 15 minutes) was followed, after an interval of an hour, by another drop of 1 mb at the same rate (over 15 minutes).

Figure 3(*a*) shows the pooled results from three experiments with *C. vicina*, each point being the mean of three experimental points. The 99.87% confidence limits for the control period (first 14 points) were obtained in the manner outlined above. The first 15-minute period of falling pressure did not significantly alter the activity level. However, a second pressure-drop an hour later resulted in a highly significant increase in the level of activity, which persisted for about half an hour. The probability that these deviations are due to chance is less than 0.13%.

It should also be mentioned that on more than one occasion, when the previous pressure trend had been quite steady for several hours, a *single* drop of 1 mb in 15 minutes was sufficient to definitely increase the activity of *C. vicina* for a short time.

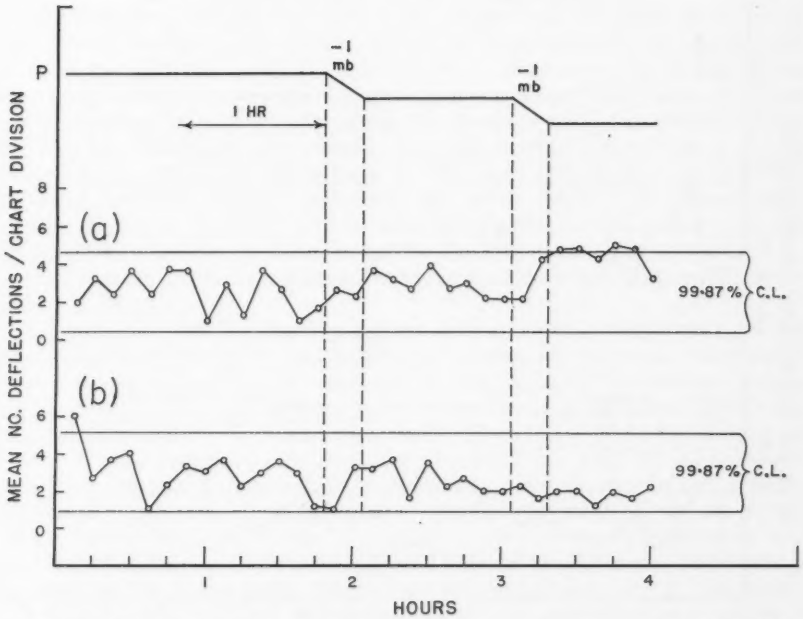

FIG. 3. Activity, from three experiments, during two step-like pressure drops (*P*) separated by 1 hour of level pressure: (*a*) *C. vicina*, (*b*) *C. vomitoria*. 99.87% limits based upon initial period of level pressure.

In contrast to the responses of *C. vicina*, Fig. 3(*b*) shows that short pressure drops had no significant effect in three experiments with *C. vomitoria*.

(*c*) *Rising Pressure*

Figure 4(*a*) illustrates, from three experiments, the effect on *C. vicina* of a change in trend from falling to rising pressure. The 2-hour period of naturally falling pressure is the control. During this period the rate of pressure drop was approximately 0.5–1 mb per hour. The activity curve continues for 1 hour into a period of artificially rising pressure, and the control confidence limits are extended through this period. The figure shows little, if any, effect of the change from falling to rising pressure. There may have been a slight depressing effect on activity following the reversal in the pressure trend, as illustrated by the single point lying below the confidence limit. However, it is unwise to place too much emphasis upon this single point.

Figure 4(*b*) shows the results of three experiments with *C. vicina* in which the pressure was *raised* in two step-like profiles, exactly the reverse of the pressure-drop experiments described in a previous section. The natural pressure trend during the initial control periods was not quite level. None of the means following the initial control period lie outside the confidence limits, and hence the two successive pressure rises had no significant effect upon activity.

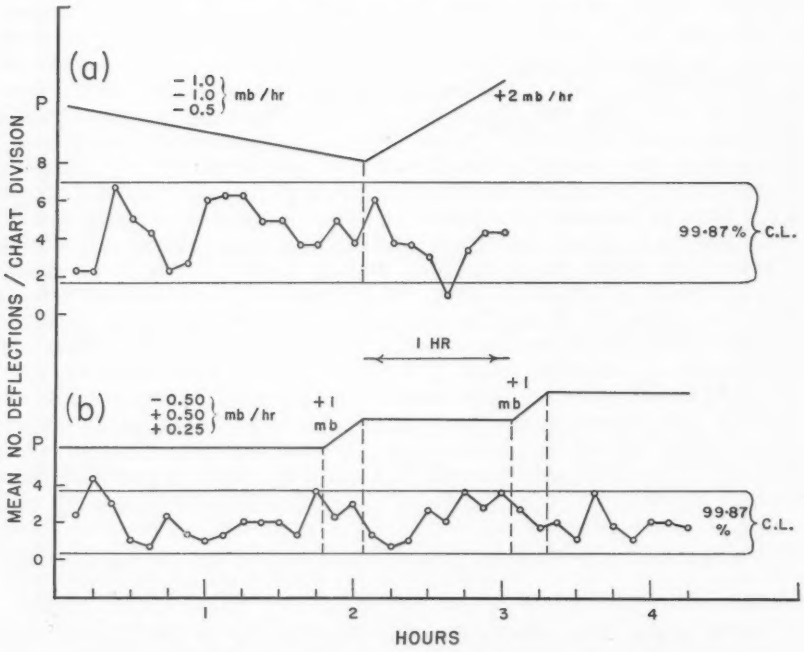

FIG. 4. Activity of *C. vicina*, from three experiments, with (*a*) steadily rising pressure following a period of steadily falling pressure (*P*), and (*b*) two successive step-like rises in pressure separated by 1 hour of level pressure. Confidence limits based upon (*a*) period of falling pressure and (*b*) initial period of level pressure.

Several experiments were carried out to observe whether rising pressure had any effect on the activity of *C. vomitoria:* no consistent effect was observed.

Discussion and Conclusions

Data have been presented showing that (*a*) a natural, or experimental, change in the trend of barometric pressure from an almost level, or a rising condition, to one of steadily, and slowly, falling pressure produced an increase in the activity of adult *Calliphora vicina;* (*b*) the activity of *C. vicina* was increased by a step-like succession of two pressure *drops*, each as small as 1 mb occurring over a period of 15 minutes, and separated by an interval of 1 hour; sometimes, increased activity resulted from only a *single* pressure drop of 1 mb; (*c*) it was the *change* to falling pressure, and not falling pressure *per se*, which stimulated activity in *C. vicina;* (*d*) *C. vicina* was little, if at all, affected by changes to *rising* pressure of the same order of magnitude as (*a*) and (*b*); (*e*) no definite effect of pressure changes on activity was observed with *C. vomitoria.*

The marked difference between *C. vicina* and *C. vomitoria* in their response to pressure changes was unexpected. In fact, the two species are so super-

ficially alike that *C. vomitoria* was at first mistaken for *C. vicina* in the laboratory. Some of the former were taken from a *new* culture and run through some of the pressure experiments in the belief that they were *vicina*. When the results of these experiments made it obvious that this group of flies was not responding to pressure changes in the previous way, the morphology of the flies was examined more closely. It was then observed that in a certain kind of light, and when viewed from a particular angle, part of the abdomen appeared to have a very slightly greenish tint, as well as the typical blue color of *C. vicina*. The insects also had black cheek-patches, rather than the reddish ones of *vicina*. At this point, some specimens were sent to the Entomology Research Institute, Ottawa, for identification, where they were identified as *C. vomitoria*.

According to Hall (9), the two species were originally considered by some authors to be synonymous, and merely environmental variations: "In nature, *vomitoria* and *vicina* seem to react in a similar fashion. The former is often larger than *vicina*, and it appears to be slower on the wing". But Wardle (9) reported *vomitoria* to be much more readily attracted to baits in shade than *vicina*. To these observations, the present author can add: (*a*) *C. vomitoria* does not react to pressure changes of the order of magnitude of those in these experiments, whereas *C. vicina* shows definite reactions; (*b*) at the local, indoor, collection source of flies, *C. vicina* was obtained up to November, but not afterward, during the winter months, whereas *vomitoria* was obtained at the same indoor source during the winter months, but not prior to December.

Wellington (18) reported that he was able to localize the site of the baroreceptor (for *sudden* pressure changes) in the aristae of several muscoid Diptera. Although part of the difference in the pressure responses of the two species reported here may be reflected in a difference in the morphology of their antennae, it is very unlikely that a localized sensory organ such as a baroreceptor would be responsible for *prolonged* activity effects. It seems more likely that a more generalized, systemic effect of lowered pressure would be involved in the prolonged increases of activity observed in *C. vicina* (e.g., about 30 minutes in Fig. 3(*a*)).

It may be useful to consider the effect of falling pressure on activity in the light of two different kinds of problems, namely, experimental studies on the activity of insects in the laboratory (e.g., upon application of external stimuli, and studies of diurnal rhythms), and effects of approaching storms on insect behavior in the field.

In laboratory studies of insect activity responses to any other stimulus, it would be desirable at least to be aware of barometric pressure changes during the experiments, through simultaneous barograph records, since there is a possibility that, with some insects, changes in the *trend* of the barometer during the experiment could influence the results. In studies of diurnal activity rhythms in insects the possible role of barometric pressure changes should definitely be considered, since a diurnal cycle of barometric pressure may influence the amount of activity.

When a frontal storm is approaching, the barometric pressure usually begins to fall, and at this time the increases in insect activity begin to be

apparent in the field. The pressure may fall fairly evenly, or, if there is much turbulence, the downward trend may be quite "bumpy". The pressure in our present location at sea level can drop for varying periods at rates comparable with those in these experiments (up to 1 mb/hour if falling steadily, or 1 mb/15 minutes if "bumpy"). The experiments have provided evidence that the often-described prestorm increase in the activity of some insect species in the field can be related to the changing trend toward falling pressure. It is probable that the increase in activity manifested at the beginning of the downward trend does not persist during the entire downward trend in pressure. In these experiments, the insects appeared to be no more active on days when the barometer was falling all through the day than during days when there was no definite trend one way or the other (Table I: July 25, 26, 28, and 29; 1000^h–1300^h). Following frontal passage, the barometer begins to rise. The present studies suggest that this reversal in trend does not affect the insects as much as the first trend downward.

It should be remembered that other factors which may be coincident with the onset of a frontal system (e.g., changes in temperature, humidity, and light) exert their own respective influences upon insect activity in the field. In addition, the density of air ions can increase outdoors during periods of falling barometric pressure, and it has been shown (*6*) that experimental air ions can increase the amount of activity of *Calliphora vicina*. In ordinary frontal passages, therefore, the complex of factors probably governs activity. But, when there is little change in the other factors during frontal approach, as sometimes happens with weak cyclonic systems, pressure changes alone can influence activity in some species.

The positive effect on *C. vicina* activity of small pressure changes also suggests another line of thought. A drop in pressure of 2 mb corresponds approximately to an increase in altitude of 60 ft. The results of the present experiments suggest that the activity of certain insects might actually be increased by slightly lowered pressure as a result of being carried to higher, but still comparatively low, altitudes (e.g., up the side of a hill or other obstacle) by air currents or by flight.

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THE IRRIGATION OF THE GILLS IN FISHES

I. STUDIES OF THE MECHANISM OF BRANCHIAL IRRIGATION[1]

RICHARD L. SAUNDERS[2]

Abstract

A technique which does not interfere with normal respiratory movements was developed for measuring the changes in hydrostatic pressure in the oral and opercular cavities of fish breathing at various intensities. This consisted of inserting polyethylene tubing of small diameter through the cleithrum into the opercular cavity and through the rostrum into the oral cavity. These cannulae were connected with manometers which were used together with optical lever systems to record the time course of changes in hydrostatic pressures in the two components of the branchial pump.

In the white sucker, brown bullhead, and carp the flow of water over the gills is nearly continuous. There are only brief periods when the pressure in the opercular cavities exceeds that in the oral cavity and therefore only brief reversals in flow in which some water may be returned over the gills. The slits between adjacent hemibranchiae of neighboring gills were observed to open rhythmically during moderate and heavy breathing. This observation is contrary to the thesis of many workers that water bathing the gills flows only through the minute pores between adjacent lamellae. Such opening of the gill slits may have a significant effect in the operation of the gills.

Introduction

Branchial irrigation, the passage of water over the gills for the purpose of respiration, is accomplished in teleosts through the active respiratory movements and as a result of the fish's forward movement through the water. This investigation is a comparative study of the active respiratory movements and the mechanisms producing them in three fresh-water teleosts. The time course of changes in hydrostatic pressure was measured and recorded, and an analysis was made of the movements of the gills themselves during their irrigation.

The Branchial Irrigation System

The Branchial Pump

The following works should be consulted by those interested in discussions of the mechanics of branchial irrigation systems of fishes: Baglioni (2), Babák (1), Leiner (16), Henschel (9, 10), Krogh (15), Black (4), Fry (5), Hughes and Shelton (14), and Hughes (12).

The anatomical mechanism for branchial irrigation in teleosts consists of the mouth and oral cavity, the pharyngeal gills, the branchiostegal apparatus, and the opercula with their enclosed cavities. Specialized muscles and skeletal elements are associated with each of the components. This mechanism moves a continuous or nearly continuous stream of water in the direction from mouth to opercular cavities. Woskoboinikoff (19) and Woskoboinikoff and Balabai (20) described the respiratory flow as being the action of two pumps: the

[1]Manuscript received May 17, 1961.
Contribution from the Ontario Fisheries Research Laboratory, Department of Zoology, University of Toronto, Toronto, Ontario.
[2]Present address: Fisheries Research Board of Canada, Biological Station, St. Andrews, N.B.

mouth and oral cavity act as a force pump pushing water over the gills, and the opercula and branchiostegal apparatus act together as a suction pump drawing water over the gills. The gills are arranged in the pharynx in such a way as to separate these two kinds of pump by forming a fine screen through which the water flows on its passage from oral to opercular cavities.

The oral cavity in teleosts is aided in its force pump capacity by one or two oral valves. These valves are classified by Gudger (6) as maxillary (dorsal) or mandibular (ventral). They are velar folds of tissue originating from the mucosa on the inner surface of either or both jaws. These structures act passively and offer no resistance to water entering the mouth as the oral cavity enlarges. As the oral cavity is contracted, the valve or valves move into position closing the opening and preventing regurgitation of water. Thereby, the water inspired is forced over the gills and into the opercular cavities.

Water is drawn from the oral cavity, over the gills and into the opercular cavities, by abduction of the opercula. Entrance of water posteriorly is prevented during this suction phase by the flexible extensions of the posterior ends of the opercula. These maintain contact with the skin covering the lateral margins of the cleithra during abduction of the opercula and function as valves. Then the opercula are adducted and their valves swing open, allowing egress of the water which has flowed over the gills.

Woskoboinikoff and Balabai (20) used water manometers to measure the changes in hydrostatic pressure occurring in the oral and opercular cavities during breathing. Later, Hughes and Shelton (13, 14) and Hughes (11, 12) used capacitance manometers together with cinematography to study the time course of pressure changes in the branchial pump of a number of species. They elucidated the relation between the maxima and minima of hydrostatic pressures and the various breathing movements. In so doing, they demonstrated that the pressure in the oral cavity is not always positive with respect to that in the opercular cavities. However, the period during which the opercular pressure exceeds that in the oral cavity is only a small fraction of the respiratory cycle. The present study considers this phenomenon with other species. Further, measurements of the amplitude and frequency of pressure changes in the oral and opercular cavities have been made during gentle breathing and various degrees of labored breathing brought on by altering the partial pressure of carbon dioxide in the water.

The Gills

The gills are supported by four pairs of bony arches arranged serially in the pharynx. Each gill arch bears two rows of gill filaments, the hemibranchiae, and individual filaments bear the secondary lamellae or respiratory plates, which provide a large respiratory surface. The tips of adjacent rows of filaments touch and the lamellae of adjacent filaments within the same row interdigitate with one another leaving small pores for the passage of water. This compact screen forms an advantageous structure for the uptake of oxygen. Bijtel (3) has emphasized that the adjacent rows of filament tips touch one another during all phases of quiet breathing and that water can pass only through the pores between adjacent lamellae.

Studies of Branchial Irrigation Systems

Van Dam (18) and Hazelhoff (8) used fine hypodermic needles inserted behind the opercular valves to extract water which had passed over the gills in order to measure the percentage utilization of oxygen. Similarly, Hughes and Shelton (13, 14) and Hughes (12) used fine hypodermic tubing inserted between the lips and behind the opercular valves to record changes in pressure in the oral and opercular cavities respectively. This technique has two disadvantages. Many fish struggle violently when even a small object is held in the way of the oral or opercular valves. It is therefore difficult or impossible to hold such cannulae in place for long periods in fish not under anesthesia. Moreover, such placement of cannulae interferes with the normal action of these membranous valves.

In the present study van Dam's (18) and Hazelhoff's (8) technique has been modified for measuring hydrostatic pressures in the oral and opercular cavities without interfering with breathing movements. The technique may be used successfully with many species. The observations resulting from the studies employing the modified technique have added to the findings of Hughes and Shelton (13, 14) and Hughes (11, 12) that pressures in the opercular cavity often exceed those in the oral cavity during a small fraction of the respiratory cycle. Moreover, the data arising during the present study made it necessary to determine whether the filament tips of adjacent hemibranchiae remain in contact during moderate and heavy breathing as Bijtel (3) maintains they do during quiet breathing.

Materials and Methods

Species studied were: white sucker, *Catostomus commersoni* (Lacépède); brown bullhead, *Ictalurus nebulosus* (LeSueur); and carp, *Cyprinus carpio* Linnaeus. These were held out of doors in an 850-liter plywood tank which was coated on the inner surfaces with black neoprene to help reduce abrasive injury to the fish. Here the temperature was maintained at 12–16° C in the winter and 15–19° C in the summer. Normal, healthy fish were selected from the stocks and brought inside for thermal acclimation. The temperature was raised to 20° C by daily increments of 1 degree. All fish used in this investigation were acclimated at 20° C, the minimum period of acclimation being 4 weeks.

USE OF CANNULAE

Cannulae were used together with segment tambours to measure changes occurring in the oral and opercular cavities during breathing. The difficulties encountered when cannulae are introduced between the lips into the oral cavity and behind the opercular valve into the opercular cavity have been indicated in the Introduction. A new technique was developed during the present study whereby these difficulties were overcome. Cannulae were inserted in the oral and opercular cavities through the tissues adjoining these structures.

Cannula Material

The material used for cannulae was Intramedic Polyethylene Tubing produced by Clay-Adams Inc., New York. This tubing is ideal for such use

because it is pliable even when cold and does not cause tissue irritation. The size used was PE 160 with an outside diameter of 0.152 mm and an inside diameter of 0.114 mm.

Operative Procedures

Specimens were removed from the acclimation tanks before feeding time on the day preceding an experiment. They were anesthetized in a 1/20,000 solution of MS 222 (tricaine methanesulphonate). The fish were immobilized in less than 3 minutes and they resumed breathing and swimming movements within 2 minutes after they had been returned to fresh water.

The Cannula for the Opercular Cavity

A small horizontal hole was made through the cleithrum by means of a No. 18 hypodermic needle which is of approximately the same outside diameter as that of the cannula to be inserted. A length of polyethylene tubing was cut obliquely to provide a sharp point to facilitate its passage through the hole in the cleithrum. Several inches of tubing were drawn through the hole while great care was taken to avoid touching the gills. The cannula was bent clear of the gills and operculum, and the pointed end cut off. A small flange was made on the end of the tube by holding it near a small flame. When the flange was pulled tightly against the posterior wall of the opercular cavity, it provided a tightly fitting placement of the cannula. This operation was easily completed in 1 minute. The placement of the cannula in the cleithra of the species studied is shown in Fig. 1.

The Cannula for the Oral Cavity

It was difficult to find a suitable location for the cannula for the oral cavity. The lips, sides, and floor of the mouth are constantly being moved as the fish breathes. This precluded placing the cannula in any of these locations. An ideal place was found to be the ethmoid region of the rostrum on the midline just anterior to the nostrils. A small hole was made vertically through the rostrum to the roof of the mouth and tubing was introduced as in the opercular cavity. A small plastic washer was put on the cannula before the end was flanged. This prevented the cannula from being pulled out. Figure 2 shows the cannula for the oral cavity in a bullhead skull.

The location of the cannula varied slightly in the three species. In suckers and carp it penetrated the space anterior to the unpaired dermethmoid and posterior to the paired maxillary bones. In the bullhead the cannula entered

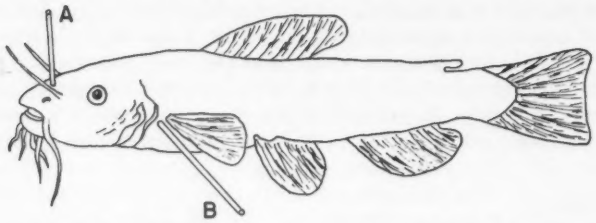

FIG. 3. Sketch of bullhead showing two cannulae in place: A, cannula to the oral cavity; B, cannula to the opercular cavity.

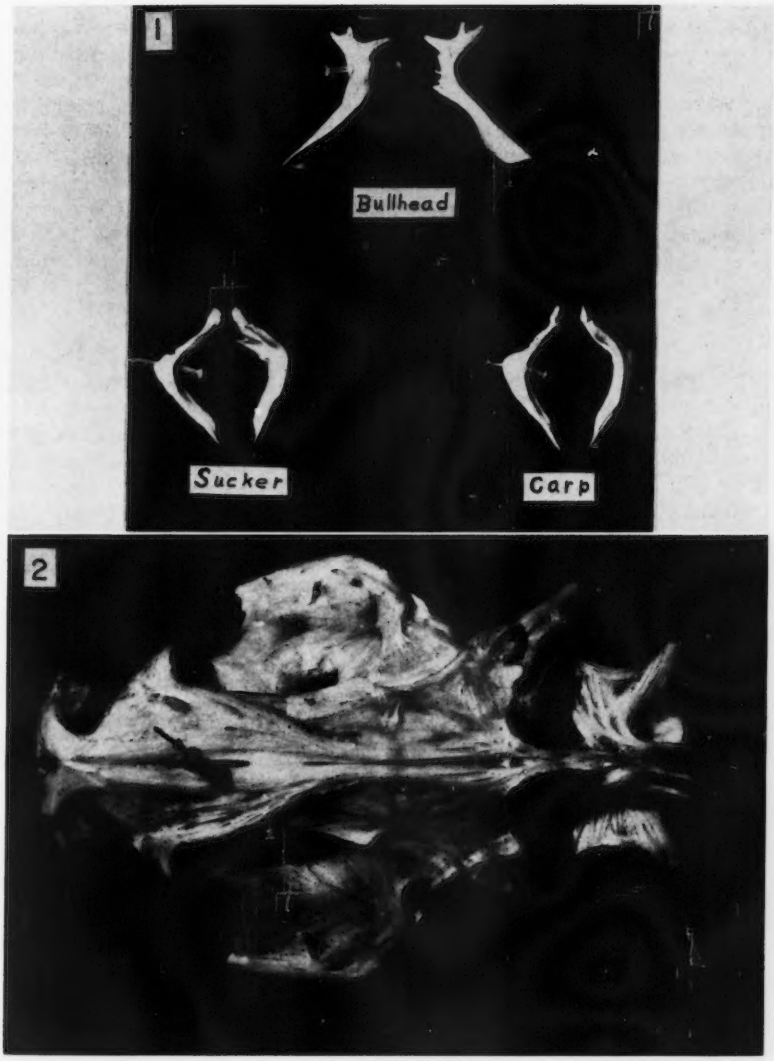

FIG. 1. Paired cleithra of bullhead, sucker, and carp with a cannula inserted in the left cleithrum of each pair.
FIG. 2. Skull of bullhead with cannula for oral cavity inserted through the rostrum.

the foramen between the paired frontals and the unpaired dermethmoid and emerged ventrally through the vomer just anterior to the parasphenoid. The breathing movements were not impeded by the cannulae fixed in these locations. Moreover, the cannulae passed well anterior to the brain and in a region believed free of cranial nerves and principal blood vessels. Figure 3 shows the two kinds of cannulae in position in a bullhead.

Following the operative procedures described above, the fish were placed in running tap water at 20° C. Here they could recover from the operation and the anesthetic. The fish were invariably resting and breathing quietly when observed next morning. The cannulae were removed after completion of experiments and the fish were returned to acclimation tanks. In most cases the small holes left after removal of the cannulae healed quickly and the fish could be used again in about 2 months.

MEASUREMENT OF CHANGES IN PRESSURE IN THE ORAL AND OPERCULAR CAVITIES

Apparatus

Direct measurements were made of changes in hydrostatic pressure in the oral and opercular cavities of fish confined in a perforated, plexiglass tube 3 in. in diameter. This tube rested on the bottom of an aquarium of 23-liter capacity which was supplied with running water in which the oxygen and carbon dioxide levels could be varied. The cannulae, leading from the oral and opercular cavities were attached by means of drawn-glass connections to quarter-inch Tygon tubing. The Tygon tubing was in turn connected to a glass tube flared slightly at the distal end and held within a glass water jacket like that shown in Fig. 4. The water jacket has two projecting arms, a horizontal one for connection with the aquarium and a vertical one for a water levelling tube and bubble trap. A piece of thin latex rubber was stretched loosely over the flared end of the glass tube and cemented in place. This operation must be done so that the resulting diaphragm has no tension, yet is without slack. This diaphragm serves as a segment tambour, which must be very sensitive to changes in hydrostatic pressure. The care exercised in attaching the latex

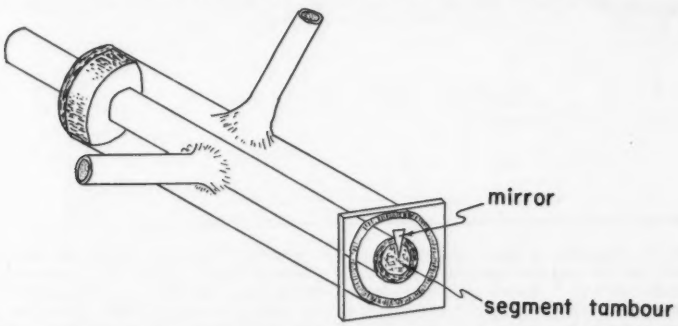

FIG. 4. Segment tambour held within water jacket. The segment tambour is separated from the flat end of the water jacket by a distance of about 1/8 of an inch. This permits unrestricted movement of the segment tambour and the mirror.

rubber ensures sensitivity to hydrostatic pressure changes, especially in the range near zero pressure. A small, triangular piece of mirrored glass, made from an aluminum-coated cover slip, was then cemented to the segment tambour. The segment tambour was held in the water jacket, which was connected to the aquarium to keep the same hydrostatic pressure acting on the outer surface of the rubber membrane as was acting on the fish in the aquarium. (The segment tambour is shown in Fig. 4.)

The connections including cannulae, glass, and Tygon tubing were kept as short as possible to reduce resistance to fluid motion. The connections from the oral and opercular cavities to their respective segment tambours were made the same length to equalize the surface resistance. The resulting system was an extremely sensitive indicator of changes in hydrostatic pressure within the oral and opercular cavities. An observer could easily count the fish's breathing movements by watching the segment tambours.

The recording system was completed by an optical lever. This pressure recording system is shown diagrammatically in Fig. 5. Separate projection lamps (10, in Fig. 5) were focused on each segment tambour (9, in Fig. 5) and the light reflected from the mirrors on the segment tambours was directed onto a plano-convex lens mounted a fixed distance in front of a variable-

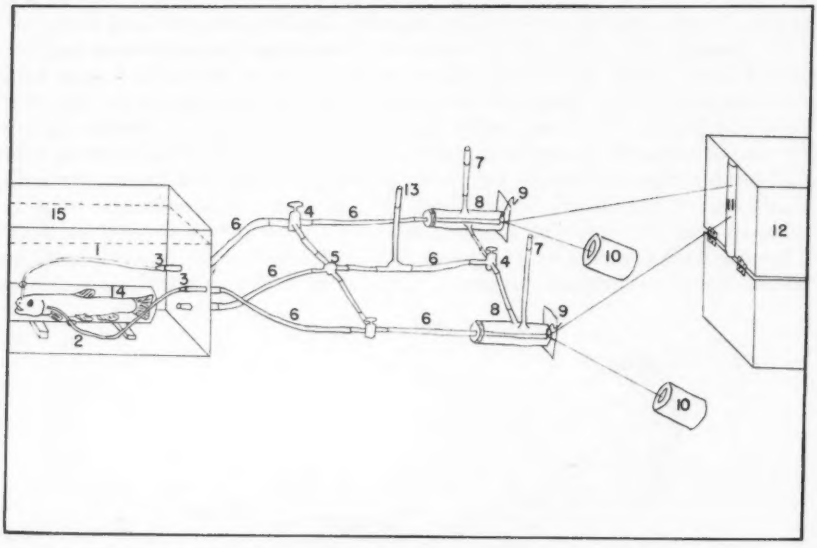

FIG. 5. Diagram of the apparatus used for recording the time course of changes in pressure in the oral and opercular cavities: 1, cannula from oral cavity; 2, cannula from opercular cavity; 3, drawn-glass adapters for connecting cannulae to larger-sized tubing; 4, three-way glass stopcocks for aligning segment tambours with the fish's branchial pump system or with the aquarium; 5, four-way glass connection; 6, Tygon tubing; 7, levelling tubes, bubble traps; 8, glass water jackets; 9, segment tambours; 10, light sources; 11, plano-convex lens; 12, hinged box containing revolving drum; 13, bubble trap; 14, perforated chamber for holding fish; 15, aquarium.

speed revolving drum. This lens was made by sawing a piece of 1-in.-diameter plexiglass rod along the median longitudinal plane. The rough surfaces were smoothed with sandpaper and the whole lens polished with jewelers' rouge and a buffing wheel. The beam of light projected on the segment tambour was made a thin, horizontal strip by passing it through a slit between two razor blades held within a frame which was mounted in front of the light source. Two strips of black tape were attached to the plane surface of the plexiglass lens leaving a narrow slit between them. The reflected light from the mirror was a thin, horizontal strip. This strip was reduced to a small point of light on the revolving drum after it passed through the vertical slit between the strips of tape.

Permanent records of pressure changes during the breathing cycle were made on Kodagraph extra thin, fast projection paper mounted on the revolving drum. The latter was geared for precise speeds of rotation of 1, 5, and 15 seconds as well as 1 and 5 minutes.

Recording Changes in Pressure

A standard procedure was adopted for recording changes in hydrostatic pressure associated with breathing. The room was darkened and the projection lamps turned on for prescribed periods after the segment tambours for the oral and opercular cavities had been connected with these cavities or with the aquarium. The segment tambours were made to register zero pressure by closing the three-way stopcocks (4, in Fig. 5) to the cannulae and opening them to the aquarium. The zero reference pressure was recorded for each segment tambour during one rotation of the drum. These reference marks were horizontal, parallel straight lines. Then the three-way stopcocks were opened to the cannulae and some breathing movements recorded with the drum stopped. The resulting records were two parallel, vertical straight lines which showed only the amplitude of the changes in pressure during the breathing cycle. The beam of light from each mirror entered the lens at a different

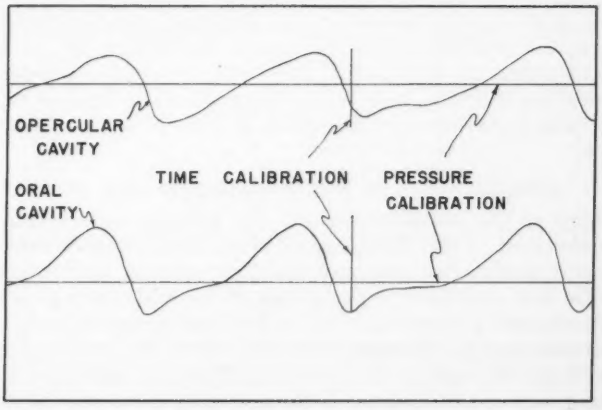

FIG. 6. Tracings from a photographic record showing time course of changes in hydrostatic pressure in the oral and opercular cavities of a white sucker. Note respective calibration lines for time and pressure.

angle with the result that the vertical lines on the record were a certain distance apart. The intersection of each vertical line with its respective horizontal line gave a point of reference which represented zero pressure and time. Finally, a number of breathing cycles were recorded with the drum rotating. A set of curves illustrating the changes in hydrostatic pressure during the breathing cycle of the sucker is given in Fig. 6.

Calibrating Segment Tambours

Since no two segment tambours had the same elasticity, the resulting pressure records made simultaneously with different tambours were not directly comparable. This difficulty was overcome by calibrating each segment tambour and then correcting one to the other. A U-shaped water manometer was attached to the levelling tube (7, in Fig. 5) by means of rubber tubing. The stopcocks were closed. A piece of paper ruled in millimeters from zero to plus and minus 25 was stuck over the vertical lens and the beam of light made to rest at zero. Then the light beam was made to come to rest at a number of places above and below the zero mark. This was done by applying pressure or suction to the open end of the U-shaped manometer. The pressure in cm of water and the corresponding deflection of the light beam in mm measured on the ruled paper were recorded for a number of deflections above and below zero deflection. The results were plotted as deflection in mm against pressure in cm of water. This procedure was repeated for each segment tambour on each day it was used.

The information gathered in this way made it possible to correct readings using one segment tambour to those with another. For example, if one tambour gave a deflection of 7.5 mm for a pressure of 10 cm of water and a second one gave a deflection of 6.2 mm for the same pressure, then the first is more sensitive than the second by a factor of 1.21. The readings from the second tambour can be made comparable with those of the first by multiplying them by 1.21. One set of curves can be made to have the same relation of deflection to pressure as another by measuring the distances from zero pressure to the curve at regular intervals along the record, multiplying by an appropriate factor, and plotting the new points. This is a very slow and tedious process. A simple drafting machine, with which this process can be done more quickly and easily, was constructed by Dr. F. E. J. Fry. It is a modification of the pantograph.

OBSERVATIONS OF THE OPENING OF GILL SLITS

Movements of the gill filaments and the opening and closing of the gill slits were observed in the three species under consideration here. The opercular valves typically open after completion of opercular abduction and remain open for the first one-third to two-thirds of the adduction phase. The gills, thus briefly exposed, are seen to move, and transitory openings appear between adjacent hemibranchiae of neighboring gills. Since the openings to the opercular chambers are very small and the gill slits remain open only briefly, this opening was recorded using cinematography with high shutter speeds. Cameras (8-mm and 16-mm) were hand-held at close range to record the opening of the gill slits.

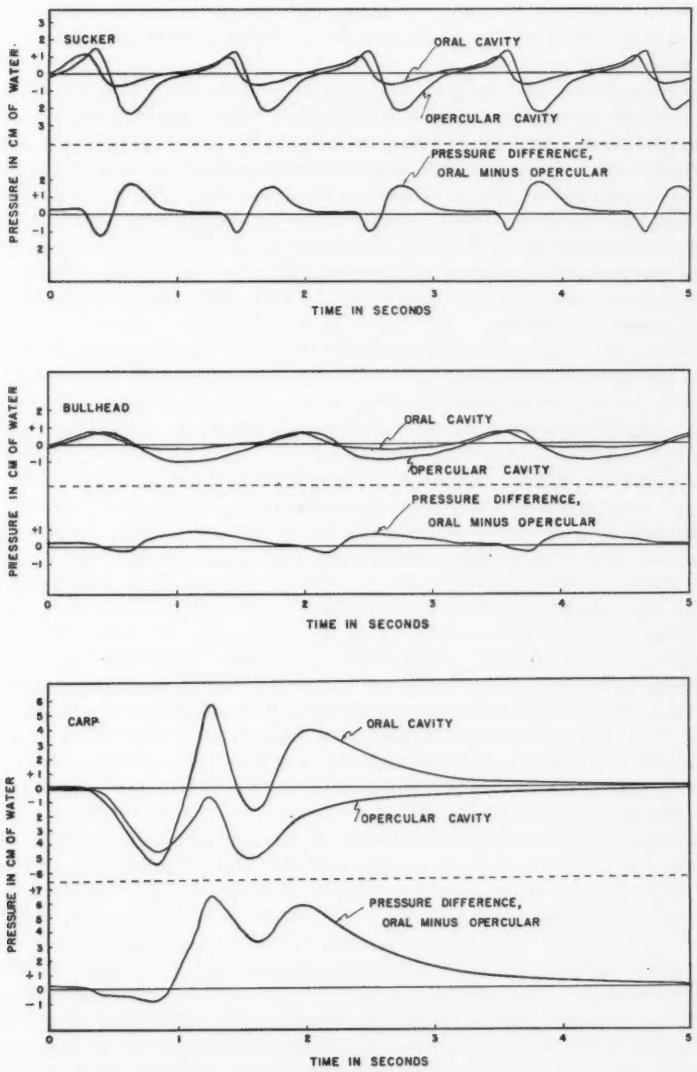

FIG. 7. Time course of changes in hydrostatic pressure in the oral and opercular cavities of sucker, bullhead, and carp during quiet breathing. These pressure curves have been reproduced from the original photographic records by the method described in the text. The curves presenting the differences in pressure between the oral and opercular cavities were obtained by measuring the vertical distances at regular intervals between the curves for pressures in these cavities and plotting the values on separate ordinate scales in the lower part of each drawing.

Results

HYDROSTATIC PRESSURES IN THE BRANCHIAL PUMP

Quiet Breathing

The time course of changes in hydrostatic pressure in the oral and opercular cavities was measured and recorded for a number of quietly breathing suckers, bullheads, and carp. These temporal changes in hydrostatic pressure are illustrated in Fig. 7, which shows some typical respiratory patterns for quietly breathing fish. These records of hydrostatic pressure in the oral and opercular cavities are plotted concurrently to show their rhythmic nature and their relations one to another. Pressures in the oral cavity were predominantly positive with respect to those in the opercular cavities. This relation between the pressures in the two cavities must exist if water is to flow from oral to opercular cavities.

One salient feature common to all three breathing patterns was a brief phase during each breathing cycle when the pressure in the opercular cavity exceeded that in the oral cavity. Such phases of back-pressure are illustrated in Fig. 7 by the curves relating differences in pressure, which were obtained by subtracting the hydrostatic pressure in the opercular cavity from that in the oral cavity. These values are plotted on separate ordinate scales below each set of pressure records. The back-pressures, if maintained, would result in a back-flow of water over the gills, but, as shown in Fig. 7, they occupy only small portions of the entire breathing cycles in bullheads and carp and a slightly larger portion of the breathing cycle of the sucker. Hughes and Shelton (14) found a similar back-pressure phase of the breathing cycles in their studies of the time course of pressure changes in brown trout (*Salmo trutta*), tench (*Tinca tinca*), and roach (*Leuciscus rutilus*). Hughes (12) extended these studies to marine fishes and found back-pressures in the breathing cycles of the following species: herring (*Clupea harengus*), whiting (*Gadus merlangus*), horse mackerel (*Trachurus trachurus*), wrasse (*Crenilabrus melops*), and five-bearded rockling (*Onos mustela*), but not in the bottom-living fishes: butterfly blenny (*Blennius ocellaris*), plaice (*Pleuronectes platessa*), and merry sole (*Microstomus kitt*).

Although there is an intermittent intake of water by the mouth and an intermittent discharge from the opercular cavities, the pressure difference curves show that the flow of water over the gills is nearly continuous. It is possible by inspection of the pressure records and their respective pressure difference curves to evaluate the relative contributions during gill irrigation of the opercular cavities as a suction pump and the oral cavity as a pressure pump. The action of the suction pump is predominant and that of the pressure pump is only of minor importance in gill irrigation of the sucker and bullhead. This is clearly shown in the records, where the maximum excursions of the pressure difference curves correspond with the maximum negative pressures recorded in the opercular cavities. Similarly, the oral cavity makes its greatest contribution as a pressure pump when the maximum excursions of the pressure difference curves correspond with the maximum positive pressures recorded in the oral cavity. Such predominance of the oral pressure pump was observed in the carp, where it contributed slightly more to gill irrigation than did the suction pump.

Outstanding differences were found in the rate of breathing and the amplitude of changes in hydrostatic pressure in the respiratory cycles of bullheads, suckers, and carp. The breathing rates of the fish described in Fig. 7 were approximately 65, 38, and 24 for sucker, bullhead, and carp respectively. These species all displayed periods of apnea when resting quietly in air-saturated water at 20° C. Bullheads occasionally suspended breathing for as long as 30 seconds; suckers rarely suspended breathing for 10–15 seconds. While resting bullheads and suckers breathe regularly with only occasional periods of apnea, carp breathing is characterized by trains of two or three rapid movements followed by an apneic period. One train of two to three respiratory movements and the accompanying apneic period lasted just 5 seconds in the example given. This alternation between breathing and apneic periods is repeated rhythmically and has been recorded continually for as long as 5 minutes in resting carp. The effect of this peculiar pattern of breathing in the carp is to build up a considerable positive pressure on the oral side of the gills. This pressure is increased during about $1\frac{3}{4}$ seconds and is gradually attenuated during the succeeding $3\frac{1}{4}$ seconds when the breathing rate is 24 per minute.

There were striking differences among the three species in the amplitude of pressure changes during breathing of individuals of equal size. The amplitude of the sucker's pressure excursions is over twice that of the bullhead, while the amplitude of breathing pressures in the carp is over twice that of the sucker.

Heavy Breathing

Heavy breathing was induced by raising the partial pressure of free carbon dioxide. The observable effects of these high levels of carbon dioxide were increases in the rate and depth of breathing. This is illustrated in Fig. 8. The breathing rates at the times these records were made were approximately 120, 50, and 110 in sucker, bullhead, and carp respectively. In all three species the rates were more regular than those usually observed during quiet breathing. There were few pauses or changes in amplitude, both of which were frequently recorded during quiet breathing. The most striking change from quiet to heavy breathing was that displayed by the carp. The slow, intermittent pattern of breathing was replaced by a rapid, regular one.

The general relations between the hydrostatic pressures in the oral and opercular cavities of the sucker and bullhead are only slightly changed from those observed in quietly breathing fish. The capacity of the opercular cavities to operate as a suction pump still appears to have the dominant action in irrigating the gills. But the comparative contribution of the pressure pump of the oral cavity is greater during this heavy breathing than during quiet breathing. The breathing patterns of heavily breathing suckers and bullheads retain the brief back-pressure phases. However, the back-pressure phases in these suckers and bullheads are of shorter duration and are lower in magnitude than those recorded during quiet breathing.

The breathing patterns of both quietly and heavily breathing bullheads and suckers differ only in small details from those recorded by Hughes and Shelton (14) for trout, tench, and roach. There is a remarkable similarity

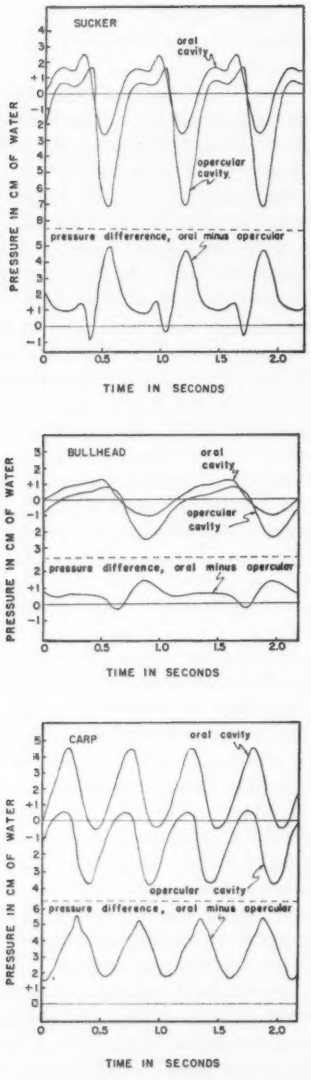

FIG. 8. Time course of changes in hydrostatic pressure in the oral and opercular cavities of sucker, bullhead, and carp during heavy breathing. The fish were induced to breathe heavily by increasing the ambient carbon dioxide level. These pressure curves were reproduced from the original photographic records by the method described in the text. The curves relating differences in pressure were obtained by measuring the vertical distance at regular intervals between the curves for pressures in the oral and opercular cavities and plotting the values on separate ordinate scales.

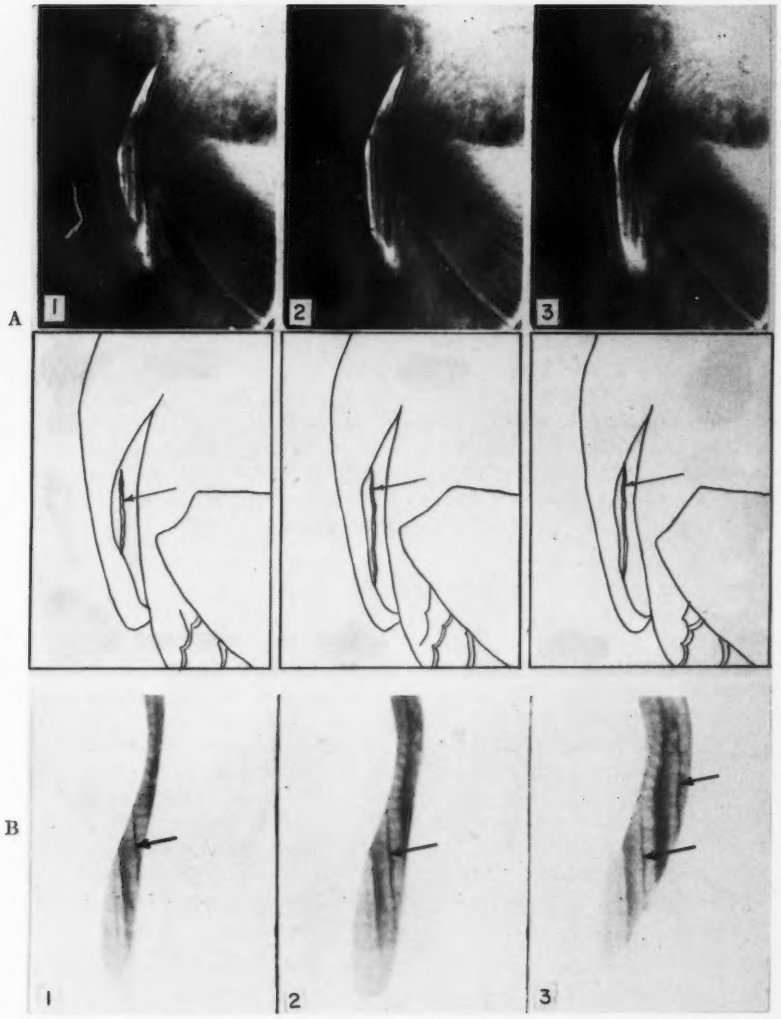

FIG. 9. Ciné photographs showing an open gill slit in a carp (A) and in a sucker (B). Arrows indicate gill slits.

A. These are negative prints enlarged from a black and white, 8-mm ciné film (positive). This sequence shows the opercular valve opening (1 and 2) and fully open (3). In each of these photographs the opening between the second and third gills appears as a white slit. The accompanying drawings are given to point out the opened gill slit in each photograph.

B. These are positive prints enlarged from a 16-mm black and white ciné film. This sequence shows the progressive opening of the opercular valve (1–3) and of two gill slits within the opercular cavity.

between Hughes' and Shelton's records of the breathing pattern in the brown trout and that of the heavily breathing sucker in the present study.

The respiratory pattern of the heavily breathing carp differs greatly from those of suckers and bullheads. There is also little similarity between the pressure curves for the carp in the present study and those of the tench and roach as recorded by Hughes and Shelton (14). All three of these latter species belong to the family Cyprinidae. In heavily breathing carp the pressure in the oral cavity remains higher than that in the opercular cavity during all phases of the respiratory cycle. Hughes (12) has recently reported a similar finding for certain marine flatfishes. Consequently, the breathing patterns of the carp and certain marine flatfishes have no back-pressure phases and therefore no reversal of flow of water over the gills.

Results of the present study indicate that the pressure relationships between the oral and opercular cavities of the branchial pump system are such that water will flow in the direction from oral to opercular cavities during most of the breathing cycle. Therefore, the gills are bathed by a continuous or nearly continuous stream of fresh water. Van Dam (18) concluded that ". . . *the water current along the gills of the rainbow trout must be continuous.* I think it very probable that the same applies to most, if not all, fish species." The present findings concerning breathing patterns in suckers, bullheads, and carp are in general agreement with van Dam's momentous conclusions.

OBSERVATIONS OF RHYTHMIC OPENING OF THE GILL SLITS

Numerous observations were made of the movements of the gills during moderate and heavy breathing. This was done by peering into the opercular cavity during the brief phase of the breathing cycle when the opercular valve is open. It was observed in moderately and heavily breathing suckers, bullheads, and carp that openings appear consistently between the hemibranchiae of adjacent gill arches during the period when the opercular valves are open. No such observations were possible when the fish were breathing quietly because the opercular valves do not open sufficiently.

A series of ciné photographs was made showing the opening of the gill slits in suckers, bullheads, and carp. Several sequences from the films showed very clearly the opening of the gill slits when the films were projected on a screen. A sequence of photographs of the head and gills of a carp and of the gills only of a sucker is shown in Fig. 9 together with drawings on which some of the details are indicated. These photographs show the open gill slits.

Discussion

OPENING OF THE GILL SLITS

An important aspect of fish breathing which has been overlooked by previous workers is the rhythmic opening of the gill slits during moderate and heavy branchial irrigation. Much of the water bathing the gills flows from the oral to the opercular cavities not through the tiny interlamellar spaces but via the openings which appear periodically between the hemibranchiae of adjacent gills. The present author has observed this phenomenon, the opening of the gill slits, in several species and believes it to have a significant effect in the operation of the gills.

Several authors (Woskoboinikoff (19), van Dam (18), Krogh (15), Bijtel (3), Hughes and Shelton (14), and Nicol (17)) have accepted as fact that the tips of filaments of adjacent hemibranchiae remain in contact throughout the normal breathing cycle and that water can pass only through the spaces between adjacent lamellae. Bijtel (3) reported, "As the gill-cover is raised for a moment in every expiration, it is possible to watch the position of the filament tips by looking under the gill-cover in this phase of breathing. Mrs. Hofdijk-Enklaar found, that in this phase of the respiration the filament tips of one hemibranchia and those of the successive one of a following branchial arch lean against each other. So during quiet respiration the aperture between two successive gills is closed in expiration." This is quite likely true during quiet breathing, when little water flows over the gills. But this idea must be rejected when heavy breathing is considered. Rhythmic opening of the gill slits was seen during the present study in most instances when the fish were breathing heavily enough for the opercular opening to widen sufficiently for an observer to see the gills. The present author was unable to observe the gills of quietly breathing fish because the opercular opening did not widen sufficiently to expose the gills.

Hughes and Shelton (14) accepted Bijtel's opinion that the gill slits remain closed during quiet respiration and tacitly accepted that this applies as well to moderate and heavy breathing. With the acceptance that the gill slits remain closed during heavy breathing, they discussed the difference in pressure which exists between the oral and opercular cavities and attempted to evaluate this in terms of gill resistance. By an experiment in which they caused a tench to vary the rate of flow of water over the gills by changing the $p(CO_2)$, they showed that the ratio of the mean pressure difference across the gills to the minute volume decreases as the minute volume and mean pressure difference increases. Hughes and Shelton reported, "It is clear, then, that under these conditions the gill resistance is variable and that it probably decreases as the pressure difference across the gills increases." They cited, as further evidence for the above conclusion, that the ratio of the mean pressure difference to the minute volume decreased as water was made to flow over the gills of a deeply anesthetized, non-breathing fish.

Observations arising during the present study permit a simple explanation for the phenomenon observed by Hughes and Shelton. Gill resistance must indeed decrease with increased opening of the gill slits. The present author reasons that the gill slits may remain closed during quiet breathing when little water flows over the gills. At higher rates of flow of water, the gill slits are either forced open by the rush of water through the pharynx or are opened actively during the breathing movements which result in flexure and contraction of the gill arches. The result of greater and greater opening of the gill slits would be a diminishing gill resistance because the water would flow through the rather large gill slits rather than through the restricted passages between adjacent lamellae.

A further consequence of opening of the gill slits is a reduction in the difference in pressure between the oral and opercular cavities and a slowing of the rush of water over the gills. This reduction in the difference in pressure between

either side of the gills could serve as an effective safety device protecting the delicate gill membranes from increased hydrostatic pressures resulting from heavy breathing. Since the difference in pressure between the oral and opercular cavities may be increased considerably in passing from quiet to heavy breathing, it is conceivable that heavy breathing could result in damage to the delicate lamellae or restriction of the flow of blood through them were there not some measure for their protection.

Opening of the gill slits could assume its greatest importance during swimming. Presumably, great volumes of water can easily be passed over the gills as a result of the fish's forward movement through the water. Although the species considered in the present investigation do not depend entirely on forward motion through the water for branchial irrigation, the mackerel does and will be asphyxiated if prevented from swimming (Hall (7)). Were the gill slits to remain closed, all the water would have to pass through the interlamellar spaces with the consequence of possible damage to the delicate lamellae. Moreover, were there no means of easing the passage of water through the gills of rapidly swimming fish, it is conceivable that there would be a considerable drag placed on the fish, thus offsetting any effects of body streamlining in overcoming resistance to motion through the water.

The continuity and magnitude of the gradient in hydrostatic pressure across the gills are important considerations in the study of the efficiency of branchial irrigation. The back-pressure phase of the breathing cycle, when water flows from opercular cavities to the oral cavity, must be detrimental to efficiency of the uptake of oxygen. However, this phase is of short duration in relation to the whole breathing cycle and is probably of minor importance. The continuity and magnitude of the positive pressure gradient in the oral cavity is illustrated in Figs. 7 and 8. Within a single respiratory cycle, the magnitude of the pressure gradient varies considerably. This would seem *a priori* to result in changing degrees of efficiency of gaseous exchange from one moment to the next during the respiratory cycle. The pressure difference curves in Figs. 7 and 8 are indicative of the volume of water passing through the interlamellar spaces. The species which maintains the most constant positive pressure gradient over the gills would appear to be the best able to take up oxygen from the water. The afore-mentioned phenomenon of opening of the gill slits could modulate the differences in hydrostatic pressure, at their upper levels, between either side of the gills in such a way as to function as a safety valve preventing too great differences in hydrostatic pressure between the oral and opercular cavities. Such a mechanism could operate to regulate the upper limit of velocity of water through the interlamellar spaces.

Summary

White suckers (*Catostomus commersoni*), brown bullheads (*Ictalurus nebulosus*), and carp (*Cyprinus carpio*) were used in these studies of the irrigation of the gills. Measurements were made of the changes in hydrostatic pressure in the branchial pump during various intensities of breathing by means of two cannulae of polyethylene tubing having a small diameter. The ends of these cannulae were fixed in the oral and opercular cavities respectively and they

were connected with segment tambours having small mirrors mounted on them. Photographic records were made of changes in hydrostatic pressure in the oral and opercular cavities by using an optical lever system.

1. The observations of the relationships of hydrostatic pressure within the branchial pump are in general agreement with those of Hughes and Shelton (13, 14) and Hughes (12), who found in other species that pressures in the oral cavity are positive with respect to those in the opercular cavities for all but a brief phase of the respiratory cycle when there is a back-pressure acting to reverse the flow of water over the gills.

2. The carp has a peculiar breathing pattern. In quietly breathing individuals the pattern consists of two or three rapid movements followed by a period of apnea. The whole cycle of breathing movements and apneic period often lasts about 5 seconds.

3. The slits between adjacent hemibranchiae of neighboring gills open rhythmically during moderate and heavy breathing. This allows some of the water to bypass the interlamellar spaces, which a number of previous workers insist provide the only course of water through the gills. The suggestion is given that opening of the gill slits is not entirely detrimental but may act as an effective safety device protecting the delicate gills from excessive hydrostatic pressures.

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NOTES ON POLYCHAETA FROM CALIFORNIA TO PERU[1]

E. BERKELEY AND C. BERKELEY

Abstract

Twenty-two species of Polychaeta are recorded, of which eleven were taken in the plankton, but some of these are certainly swimming forms of benthic species. The remainder were either dredged, collected intertidally, or by diving. Thirteen species come from Peru, six from California, and one each from Mexico, Panama, and Cocos Island. Three of them are new to science.

Introduction

This paper is essentially a continuation of our contribution of 1960 (9) on Polychaeta from the same region, except that this is now extended to Peru. Two of the collectors who sent us the material which formed the subject of the previous paper, Mr. W. L. Klawe, of the Inter-American Tuna Commission, and Mr. H. Arai, formerly of the University of California, Los Angeles, have contributed to the present collection. Dr. M. Lieberman, of the University of California College of Engineering, Richmond, California, who is now in charge of the invertebrate investigations started by Mr. W. A. Newman in San Francisco Bay, supplied us with further examples from that region, and Mr. J. H. McLean, of the Hopkins Marine Station, Pacific Grove, California, has sent us material, collected in the course of diving operations, from Carmel Canyon. Lastly, we received an interesting collection of pelagic specimens taken off the coast of Peru from Dr. P. N. Sund, of the University of Washington, Seattle. We are grateful for having been privileged to examine these collections. All of them included species which already have been recorded and adequately described from the region dealt with in this paper and, therefore, these are omitted here. We include only those species which are either new to science or have other noteworthy characteristics. The individual collectors are indicated in the text by initials, as in our previous paper.

Polynoidae

Malmgrenia nesiotes (Chamberlin). Chamberlin (11), as *Polynoe*, Hartman (19) (revision of type)

A single specimen in two pieces, measuring jointly 8 mm in length, agrees completely with Chamberlin's (11) description of the type. Collected intertidally at Mancora, Peru (W.L.K.). Hartman, on re-examining the type, assigns it to *Malmgrenia*, in which we concur.

The prostomium was so "shrunken" in the type that Chamberlain could not fully describe it. It is considerably longer than wide. The anterior, and much larger, pair of eyes are on the median transverse line and the posterior pair on the posterior margin. This is the first record of the species since the original one, which was from Santa Margarita Island, Lower California.

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Contribution from the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

Sigalionidae

Pholoë minuta (Fabricius). Fauvel (15)

A single example from San Francisco Bay, dredged in 20 ft (M.L.), measures about 12 mm and consists of 51 setigers. This is the first record of the species on the west coast of North America south of Oregon (23).

Pettibone (29) regards *P. tuberculata* Southern as a synonym of this species. We had formerly (4) doubted the synonymy. Southward (33) has shown in collections from the Isle of Man that the characters on which differentiation had been based intergrade, and we have recently confirmed this in a large gathering from the Arctic.

Tomopteridae

Tomopteris cavallii Rosa. Fauvel (15), Dales (13)

Two specimens from 4° 31′ S. lat., 82° 18′ W. long. in a tow at 15 m (P.N.S.) agree with Fauvel's and Dales' descriptions and with specimens recorded by us (7) from the coast of British Columbia. Dales (13) found the species widely, but sparsely, distributed in surface waters off the Californian coast. The present records are from the coast of Peru near the northern extremity. Rosa found it near Valparaiso, Chile. The distribution in the northeast Pacific is thus a wide one.

Nereidae

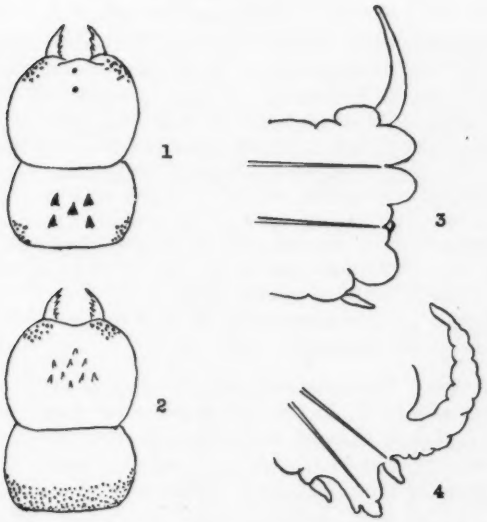
Platynereis polyscalma Chamberlin. Chamberlin (11), Hartman (20)

A number of specimens of the atokous form of this species collected intertidally at Mancora, Peru (W.L.K.). This was first described from Lower California by Hartman (20) and has not since been recorded. Epitokes have been taken from southerly latitudes, but the present record extends the known distribution of the atokous form.

Nereis* (*Neanthes*) *mancorae n. sp.

Six specimens varying in length from 5 to 20 mm and from 0.5 to 1 mm wide, from Mancora, Peru. Intertidal (W.L.K.). No color remains in the specimens. The prostomium is longer than wide. It carries two pairs of eyes, the anterior pair the larger, both pairs posterior to the median transverse line. There are three pairs of short tentacular cirri and a fourth pair (the most posterior) more than twice as long, reaching to the fifth setiger. The jaws have 9 or 10 teeth. Paragnaths as follows: I = 2 medium-sized cones in tandem; II, III, IV = 5 or 6 irregular groups of variable teeth more or less in lines; V = 1 large cone; VI = 2 large cones in tandem; VII and VIII = 5 or 6 irregular rows of small cones (Figs. 1 and 2). The dorsal cirri extend well beyond the lobes of the feet throughout the body (Fig. 3), and towards the posterior end become still longer and very heavy (Fig. 4). The parapodia have two lobes in each ramus. In the posterior region they are, however, much modified (Fig. 4). Notopodial setae throughout the body are all homogomph spinigers; in the neuropodium there are a few heterogomph spinigers in the dorsal bundle, the remainder being falcigers with finely toothed blades.

The form is differentiated from other species of *Neanthes* by the arrangement of the paragnaths and the very long and heavy dorsal cirri in the posterior region.



FIGS. 1–4. *Nereis* (*Neanthes*) *mancorae* n. sp. Fig. 1. Proboscis, dorsal view. Fig. 2. Proboscis, ventral view. Fig. 3. Median parapodium. Fig. 4. Posterior parapodium.

Phyllodocidae

Phyllodoce (*Anaitides*) *groenlandica* Oersted. Fauvel (15)

A single specimen taken over kelp beds at a night light at La Jolla, S. California (W.L.K.). This is a typically northern form and has not been recorded previously from California. Treadwell (34), however, records it from Coiba Island, Panama. His specimen was also taken at a night light.

Lopadorhynchus brevis Grube. Wesenberg-Lund (37), Berkeley and Berkeley (8)

A single specimen taken at 8° 00′ N. lat., 91° 32′ W, long. (W.L.K.). We have already recorded the species from approximately the same locality (8), as also does Treadwell (35, as *L. nans* Chamberlin). Chamberlin's record of *L. parvum* (11), which may be the juvenile of *L. brevis* (13), is from a more northerly latitude. Dales (13) records the species from California and summarizes its Atlantic distribution.

Pontodora pelagica Greef. Fauvel (15), Ushakov (36)

A single specimen collected in a 15 m tow at 9° 22′ S. lat., 79° 5′ W. long. (P.N.S.). We recorded the species (10) a considerable distance off the coast of British Columbia (50° 00′ N., 145° 00′ W.) in a vertical haul from 1250 m. The present seems to be the northernmost record, south of that one, at which it has been found. Chamberlin's (11) and Treadwell's (35) records of *Torrea pelagica* (which is a synonym of the present species) are from considerably farther south.

Alciopidae

Torrea candida (Delle Chiaje). Fauvel (15, as *Asterope*)

A small specimen, about 50 mm long as preserved, and several fragments, taken at a night light off Cocos Island (approximately 4° N. lat., 88° W. long.) (W.L.K.). This species is common in the Atlantic and Mediterranean. Dales (13) found it widely, but sparsely, distributed in the northeast Pacific, but not so far south as the present specimens. It does not seem to be known from the south Pacific.

Plotohelmis capitata (Greef). Fauvel (15, as *Rhynchonerella fulgens*)

This is by far the commonest species in Dr. Sund's collection from Peru. It occurs in 10 of his 17 stations which extend from 4° to 9° S. lat. and from 78° to 82° W. long. It has not been recorded from the northeast Pacific where it is replaced by *P. tenuis* Apstein (7, 12, 13).

Callizona nasuta Greef. Wesenberg-Lund (37), Fauvel (15)

Specimens from several of Dr. Sund's stations off Peru, mostly fragmentary, the largest complete example measuring 35 mm. The species is said to be rare in the Atlantic and Mediterranean and the only previous records in the Pacific are those of Izuka (25, as *C. japonica*) and ourselves (10).

Callizona möbii (Apstein). Dales (13, as *Rhynchonerella*)

Two specimens, incomplete posteriorly, each about 20 mm long and 0.5 mm wide, consisting of approximately 50 segments, from 10° 00′ S. lat., 78° 00′ W. long. (P.N.S.). Very little color remains, the intersegmental glands being almost colorless. The first setiger has five simple, curved acicular setae, the second four, and the third three. More posteriorly they are straight and fewer. The species has been reported from the Mediterranean (1) and north Atlantic (37). Dales records it from the northeast Pacific (13, as *Rhynchonerella*). The present record is the first from the south Pacific.

Pilargiidae

Ancistrosyllis bassi Hartman. Hartman (21)

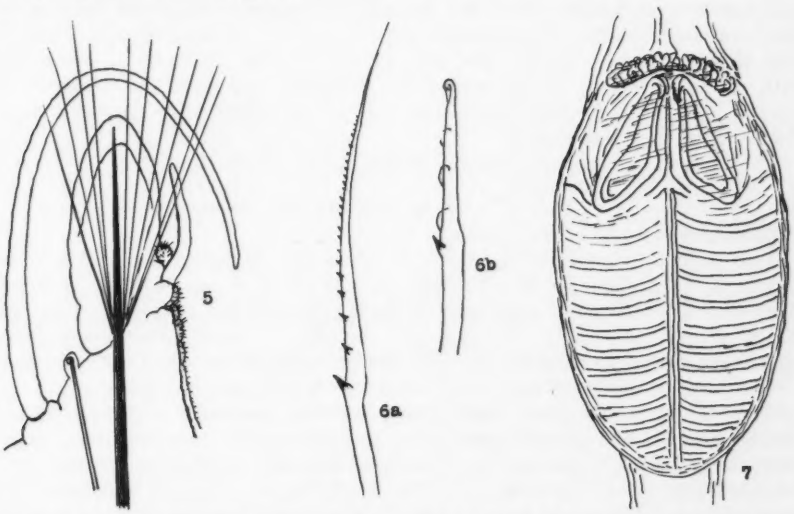
A single specimen dredged in 20 ft in San Francisco Bay, N. California (M.L.). This is the second record from this locality, which was one of the first from which one of the original specimens was obtained (21). It has since been taken in San Pedro Basin (22) and in Newport Bay (2), both in S. California.

Ancistrosyllis longicirrata n. sp.

A single specimen 10 mm long and about 1 mm wide (excluding setae), consisting of 35 segments, from 5° 55′ S. lat., 82° 2′ W. long. (P.N.S.). The prostomium is notched anteriorly, thickened dorsally, and rounded laterally. It bears three antennae, the median four times the length of the prostomium, the laterals about half the length of the median one; and has a pair of rather large eyes placed laterally close to the posterior edge. The palps are biarticulate, the palpostyles small.

The peristomium carries the first pair of tentacular cirri; the second segment (first setiger) the second pair; all are approximately the same length,

about equal to that of the median antenna. At the third segment the parapodia are almost fully developed. Thereafter their length is about that of the body-width and their width at the base about a sixth of their length. The most conspicuous feature of the parapodium is the great length of the dorsal cirrus, more than twice that of the parapodium itself. The length of the ventral cirrus is only about a fifth that of the dorsal cirrus. The parapodium is subbiramous, the notopodium a very small lobe. There is a row of ciliated prominences on the lower edge of the foot which terminates in a rather large stalked ciliated papilla emerging between the ventral cirrus and the base of the parapodial lobes (Fig. 5).



FIGS. 5–7. *Ancistrosyllis longicirrata* n. sp. Fig. 5. Median parapodium. Fig. 6*a*. Seta from dorsal side of group. Fig. 6*b*. Seta from ventral side of group. Fig. 7. Pharynx.

Setae are confined to the ventral ramus and project between terminal lobes. All are simple. The majority are very long and fine with a thickening halfway up the shaft, beyond which are serrations, the first of which are heavy and widely spaced (Fig. 6*a*). On the ventral side of the group there are three to five shorter setae which terminate in small hooks (Fig. 6*b*). The neuraciculum is heavy and protrudes with the setae. The notaciculum is much finer and ends in a small, round hook. Neither large heavy hooks nor heavy spines, such as appear, dorsal to the dorsal cirrus, in most species of *Ancistrosyllis*, are present. The pygidium ends in three small lobes, the median of which bears a pair of slender cirri. There is a well-developed muscular pharynx which encloses a pair of processes which appear to be jaws. (The detailed structure could not be made out without dissection.) Its anterior end terminates in a ring of papillae (Fig. 7).

The species of *Ancistrosyllis* hitherto described (except the extremely small and problematical *A. cingulata* of Korschelt (15, p. 251)) have been benthic.

The occurrence of the present species in the plankton and the presence of ciliated pads on the parapodia suggest that it may be a juvenile of a bottom-living form. It clearly belongs to the group characterized by long appendages (*A. constricta*, *A. robusta*, *A. tentaculata*, and *A. bassi*), but in none of these do the appendages attain the length of those of the present species, nor have jaw-like structures been described in any of them. Moreover, the form of the setae and the absence of dorsal spines are characteristics by which the present species is differentiated.

We follow Hartman (21) rather hesitantly in placing the genus *Ancistrosyllis* in the family Pilargiidae rather than in the Hesionidae, with which it was formerly classified. Hartman regards the transfer desirable because in *Ancistrosyllis* the setae are all simple, while in other genera ascribed to Hesionidae this is not the case. In the present species this character is combined with others, the long dorsal cirrus, the subbiramous foot, the presence of "jaws", and the absence of dorsal spines or hooks, which seem to ally it more closely with the Hesionidae.

The type remains for the present in the authors' collection.

Spionidae

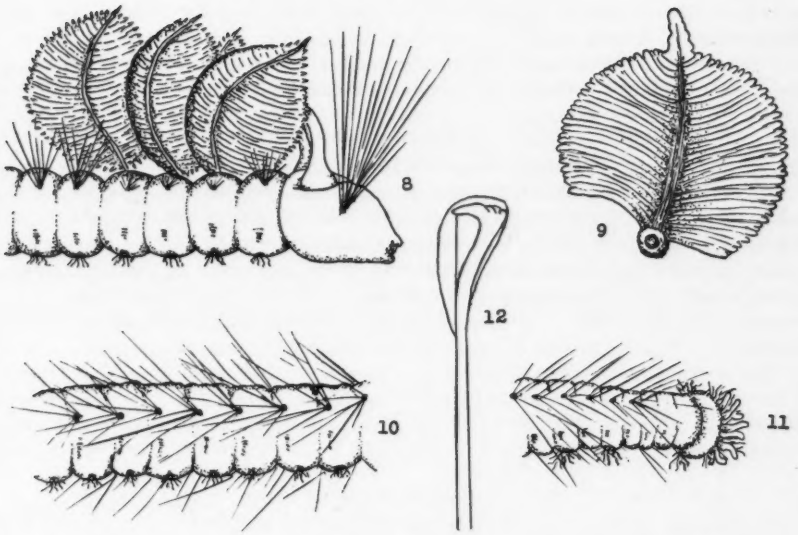
Prionospio ornata n. sp. Larvae

Two collections, consisting jointly of about 75 examples, taken in a 15 m tow at 8° 17′ S. lat., 79° 09′ W. long., and at 8° 50′ S. lat., 78° 50′ W. long. Also several incomplete specimens and fragments from 10° 36′ S. lat., 78° 02′ W. long. (P.N.S.). In all these cases the collector states that many more specimens were present in the plankton samples from which they were isolated.

The longest complete individual measures 8 mm and has 48 setigers, the average length being about 5 mm. They are thus unusually large for Spionid larvae (cf. 18). The general body color, as preserved, is a uniform, very pale, red. There are no markings. On the dorsal side the prostomium is seen as a rounded projection carrying a pair of small, dark, eyespots, but these are covered in a lateral view by the extension forward of the peristomium which bears a pair of grooved tentacular cirri and the first bunch of long larval setae. From the 2nd to the 8th setiger there are dorsally on each side a bunch of larval setae and, ventrally, of short, coarser capillaries. Branchiae are carried on the 2nd, 3rd, and 4th setigers (Fig. 8). These are the outstandingly distinctive characters of the species. Each branchia has two double rows of pinnae, which at their longest are so long that the branchia, when extended and of maximum size, is almost circular in outline (Fig. 9). The pinnae of adjacent branchiae tend to intersperse, giving the whole branchial region a striking bushy appearance.

Posteriorly to the 9th setiger and to the end of the body there are bunches of larval setae dorsally and both crotchets and short capillaries ventrally. The body surface is lightly ciliated throughout and, in addition, there are heavily ciliated protuberances ventrally (Fig. 10). The anal extremity is surrounded by a ring of cirri (Fig. 11). The crotchets are of the characteristic *Prionospio* type with a crest of three small teeth (Fig. 12).

We feel justified in designating these larval forms as a new species despite the fact that we do not know the corresponding adults, because the form of



FIGS. 8–12. *Prionospio ornata* n. sp. Fig. 8. Anterior region. Fig. 9. Branchia. Fig. 10. Median region. Fig. 11. Posterior region. Fig. 12. Crotchet.

the branchiae appears to be unlike that of any recorded *Prionospio*, adult or larval. This character seems to vary very little as between adult and larval Spionids (cf. 18). Moreover the size with maintenance of larval characters is outstanding among recorded Spionid species. A comparison of the characters of the present specimens with those of adult species of *Prionospio* suggests *P. pinnata* as the nearest ally, but the two species are differentiated not only by the form of the branchiae, but also by the absence of the vertical extension of the flaps of the peristomium and its fusion dorsally with the first setiger which characterize *P. pinnata*. So far as we know the larva of the latter species has not been described.

Cirratulidae

Audouinia polytricha (Schmarda). Ehlers (14), Monro (26, 27)

Three specimens, the first 75 mm long and 4 mm wide, the second the same length but 6 mm wide, the third 85 mm long and 6 mm wide. All collected at San Carlos Bay, Sonora, Mexico, intertidally (H.A.).

We agree with Monro (26) in regarding Chamberlin's (11) *Audouinia filigera nesophila* as a synonym of *A. polytricha*. The species agrees with *A. tentaculata* (Montagu), which has been recorded from the same general neighborhood as the present specimens by Rioja (32), in many respects, but differs from it in regard to the incidence of the branchiae and in the presence of finely toothed, curved capillaries throughout the body in *A. polytricha* but not in *A. tentaculata*. This latter character affords the most readily applied differentiation between the present species and most other

members of the genus. These capillaries are described by Ehlers (14) and figured for *A. filigera nesophila* by Chamberlin (11).

Ehlers recorded the species from the coast of Chile, Monro from Panama. The present record extends its distribution northward.

Terebellidae

Nicolea zostericola (Oersted). Fauvel (16)

Three specimens about 6 mm long and 1 mm wide in the anterior region taken in a plankton tow in 15 m at 5° 55′ S. lat., 82° 20′ W. long. (P.N.S.). Though small, they agree with adult specimens except in the presence of three or four short anal cirri. This may be a postlarval character. On the other hand, eggs are present in the bodies, but Herpin (24) has shown that sexual products occur in *N. zostericola* considerably before the adult stage is reached.

The species has not been recorded from the south Pacific, but *N. profundi* Chamberlin (11), taken in 1036 fathoms in the same region as the present specimens, approximates to it so far as can be judged from the incomplete description. It differs, however, in having 17, instead of 15, thoracic setigers.

Sabellidae

Branchiomma roulei Gravier. Gravier (17)

Three examples from Mancora, Peru, collected intertidally (W.L.K.). Other than our own record from Corona del Mar, S. California (3), this species does not seem to have been noted from the Pacific since Gravier's original description (17), from Paita, Peru, in the vicinity of the place of origin of the present specimens.

Bispira volutacornis (Montagu). Fauvel (16), Rioja (31)

Three small specimens in soft, muddy tubes from 110 ft in Carmel Canyon, S. California (J.H.M.). They agree with Fauvel's (16) and Rioja's (31) descriptions except that the ventral lobes of the collar are flattened and extended forward, possibly as a result of fixation in their tubes. The species is new to the eastern Pacific. It differs from *Volutacornis pacifica*, described by us from the coast of British Columbia (6, as *Bispira volutacornis* var. *pacifica*), in the formation of the collar.

Serpulidae

Vermiliopsis infundibulum (Phillipi). Rioja (31), Fauvel (16)

Metavermilia multiannulata Moore. Moore (28), Rioja (32, as *Vermiliopsis*)

Several examples, from Carmel Canyon, S. California, in 80–160 ft (J.H.M.) agree with the descriptions of this species. They are about 20 mm long without the branchiae, and consist of about 80 segments. The operculum, with its multiannular "chitinous" cap, is the most outstanding and distinctive feature. Specimens with a dozen, or more, opercular plates occur in the collection, but the terminal ones are readily broken off. The large, flaring collar segment is also very characteristic. The thoracic membrane is folded rather closely over the thorax in the present specimens, doubtless because of their having been preserved in their tubes.

The species has not been recorded from the Pacific as *V. infundibulum*, but we can see no reason to doubt its identity with *Metavermillia multiannulata* (Moore) (28). Rioja's redescription (32) confirms this synonymy. He mentions certain differences in tube formation between his specimens from Mexico and Moore's from S. California, but Moore's description of it is very brief and based upon a fragment. In the present collection there is considerable variation between the tubes. In general they share the characters shown by Fauvel (16, fig. 124*c*) for *V. infundibulum* and those of the relatively smooth tube shown by Rioja (32, pl. 9, fig. 28) for *V. multiannulata*. Most of them are heavily transversely wrinkled, have two or more irregular longitudinal ridges, one or two wide flanges, and a flaring mouth. In some cases two or more tubes were adherent to one another, suggesting that they had grown under crowded conditions.

The species is known from the Atlantic, Mediterranean, and S. California.

Chitinopoma groenlandica (Mörch). Pixell (30), Berkeley and Berkeley (5)

Several specimens of this species, collected at approximately 80 ft in Carmel Canyon, S. California (J.H.M.), agree with Pixell's (30) and our own descriptions. Most previous records of it (frequently under the synonym *C. fabricii* Levinsen) are from northern latitudes. From the northeast Pacific it is known only from Vancouver Island (30, 5).

Paradexiospira vitreus (Fabricius). Pixell (30), Berkeley and Berkeley (5)

Many specimens on shells collected in approximately 80 ft in Carmel Canyon, S. California (J.H.M.). A typically northern species. Not recorded previously on the west coast of North America south of Vancouver Island (30, 5).

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THE MONONCHIDAE: A FAMILY OF PREDACEOUS NEMATODES

I. GENUS MYLONCHULUS (ENOPLIDA: MONONCHIDAE)[1]

ROLAND H. MULVEY

Abstract

A resumé of the genus *Mylonchulus* is presented, including three new species which are described and illustrated, *Mylonchulus prodenticulatus*, *M. sigmaturellus*, and *M. solus*. Males of *M. brevicaudatus*, *M. incurvus*, *M. index*, *M. montanus*, and *M. striatus* are described and figured for the first time. Several known species from various parts of the world are redescribed and figured. Relationship of amphid aperture width to the length and width of the buccal cavity and its position in this genus was studied and evaluated for use in taxonomy. Two taxonomic keys are included, one of all known females, the other of all known males.

Introduction

The present paper is the first in a projected series on the taxonomy of the family Mononchidae and deals with all the known species of the genus *Mylonchulus* (Cobb, 1916) Altherr, 1953.[2]

The mononchs are a group of free-living predatory nematodes that inhabit soil and fresh water where they feed on small animal organisms, including protozoa, rotifers, and other nematodes. They are beneficial to man in that they destroy nematodes which are injurious to agricultural crops. Thorne (47), after extensive study of several species of Mononchidae in Utah, U.S.A., soils, concluded that *Mononchus papillatus* Bastian, 1865 would be of considerable aid in the control of the sugar-beet nematode (*Heterodera schachtii* Schmidt, 1871), if it were possible to maintain large populations of this mononch in the infested fields. Cassidy (9) observed that *Iotonchus brachylaimus* (Cobb, 1917) Andrássy, 1958 in culture devoured large numbers of *Heterodera* eggs and larvae. Steiner and Heinly (45) reported that a single *M. papillatus* killed 1332 nematodes in a period of 12 weeks.

I have observed that *M. papillatus* sometimes swallows the nematode whole. This is accomplished by the head end of the victim being sucked in first, followed by a sucking movement of the esophagus and the mouth as more of the nematode is engulfed. The captured nematode is partly regurgitated each time the sucking movement is conducted. The whole esophagus moves backwards and fowards with the intestine until all the victim is swallowed. In Fig. 1 *Anatonchus tridentatus* (de Man, 1876) Altherr, 1953 is shown in the act of devouring a nematode whole.

In some instances the cuticle of the devoured nematode is voided from the intestine through the anal opening, which suggests that the captor has been unable to utilize this part of the prey. Sometimes only the body contents are sucked from the victim (Fig. 2) and the skin is cast aside.

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Contribution from the Nematology Section, Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.

[2]At the present time I consider Altherr, 1953 has precedence over Pennak, 1953 as the authority for the genus *Mylonchulus*.

Materials and Methods

The study is based on preserved specimens borrowed from other institutions and on fresh material taken from soil samples collected from various parts of Canada over a number of years. The nematodes were taken from the soil by screening and then these were killed by the judicious application of heat. Some of these specimens were then examined in water mounts while others were fixed in 5% formol for at least 24 hours and then stained in either 0.05% cotton blue or 0.05% acid fuchsin in lactophenol. Permanent mounts, in either lactophenol or glycerin (6), were then prepared. Specimens taken from intercepted material from Europe, and from other foreign countries, were prepared in the same manner as described above. Head mounts were prepared in the manner described by Goodey (21) except that Thorne's Zut was used in sealing the cover slip.

Measurements were made from camera lucida drawings of each specimen using the de Man formula in all cases. Buccal cavity measurements do not include the lip or vestibule leading into the cavity. Tail measurements were calculated by drawing a line from the tip of the tail through the middle area and then projecting from the anus a line at right angles to this mid-line.

Characters Used in Species Separation

These include size of dorsal tooth, size of buccal cavity and prominence of striations in its walls, thickness of buccal cavity walls, position and size of submedian teeth, size and number of rows of denticles, lip shape, position of vulva and number of gonads, presence or absence of caudal glands, position of spinneret opening, and shape and length of tail. Body length does not appear to be a reliable criterion for species separation because of considerable variation within the species. Specimens from the southern hemisphere tend to be shorter than those from the northern hemisphere. Known species in the

TABLE I

Position of amphid aperture with respect to apex of dorsal tooth in *Mylonchulus* spp.

Species		Number with various positions of amphid aperture					Total no. of specimens
		Well forward	Slightly forward	At level of apex	Slightly posterior	Mid-way	
brevicaudatus	(females)	7	5	6	2	—	20
	(males)	2	—	1	1	—	4
montanus	(females)	6	8	3	1	2	20
	(males)	2	—	—	—	—	2
brachyuris	(females)	1	—	10	12	2	25
sigmaturus	(females)	—	3	8	9	—	20
incurvus	(females and males)	—	2	4	2	3	11
sigmaturellus	(females and male)	—	—	—	3	2	5
index	(females and male)	—	—	—	3	3	6
lacustris	(females)	5	—	3	—	1	9
solus	(female)	—	—	1	—	—	1
prodenticulatus	(male)	—	—	—	1	—	1

PLATE I

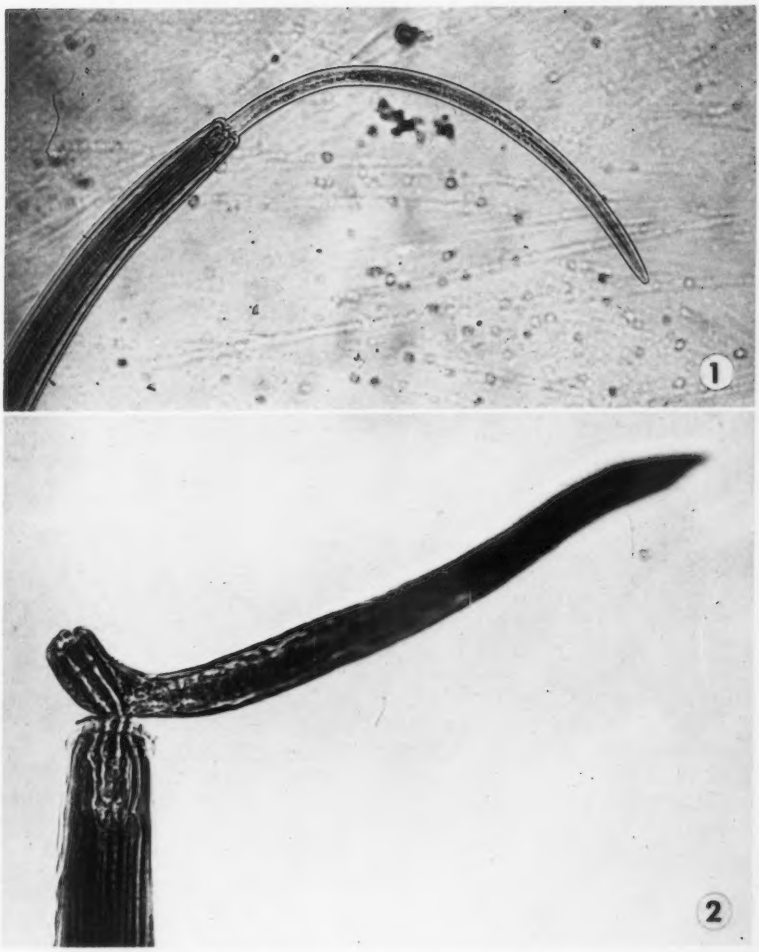


FIG. 1. *Anatonchus tridentatus* devouring another nematode.
FIG. 2. *Mononchus papillatus* sucking the body contents from another nematode.

Mulvey—Can. J. Zool.

TABLE II

Relationship of amphid aperture width of some *Mylonchulus* spp. to body length, and buccal cavity length and width

Species	Locality	No. of specimens	Average body length (mm) (A)	Buccal cavity		Amphid width (μ) (D)	Percentage			
				Av. length (μ) (B)	Av. width (μ) (C)		D/A	D/B	D/C	D/B × D/C
brevicaudatus	Canada	20 ♀♀	1.5 (1.3–1.8)	32.5 (30–36)	18.5 (15–20)	3.3 (2.8–4.0)	0.22	10.2	18.0	181 (134–240)
brevicaudatus	Canada	4 ♂♂	1.6 (1.4–1.7)	33.2 (32–35)	18.0 (16–19)	3.5 (3.0–4.0)	0.22	10.2	19.5	202 (144–264)
rotundicaudatus	Canada	1 ♀	1.7	35	22	4.2	0.25	12.0	19.0	228
solus	Canada	1 ♀	1.4	34	18	3.8	0.27	11.0	21.0	231
montanus	Canada	20 ♀♀	1.7 (1.5–1.9)	33.8 (31–35)	20.8 (20–23)	4.7 (4.0–5.0)	0.27	14.0	22.6	318 (247–384)
montanus	Canada	2 ♂♂	1.5–1.6	30–33	20–21	5.0	0.31	16.0	24	360–408
lacustris	U.S.A. (Florida, Missouri)	3 ♀♀	1.17 (1.0–1.2)	20–22	12–14	3.7 (3.7–3.8)	0.35	17.3	28.6	496 (459–527)
incurvus	Canada	8 ♀♀,	2.0	36.0	20.8	6.4	0.33	17.9	31.2	577
incurvus	U.S.A. (Calif.)	3 ♂♂	(1.5–2.5)	(30–38)	(17–23)	(5.1–8.0)				(322–924)
brachyuris	Canada	25 ♀♀	1.2 (1.1–1.5)	22.1 (20–24)	13.1 (12–16)	4.2 (3.7–4.8)	0.33	18.1	30.6	608 (408–680)
brachyuris	Canada	3 ♂♂	1.20 (1.0–1.4)	21.5 (20–23)	13.0 (12–15)	4.3 (4.0–5.0)	0.37	20.2	34.0	688 (608–726)
sigmaturus	U.S.A.	20 ♀♀	1.3 (1.1–1.9)	24.6 (23–31)	14.9 (14–20)	4.8 (4.2–6.3)	0.36	18.6	32.3	637 (510–814)
sigmaturus	Sicily, Italy	6 ♀♀	1.1 (0.9–1.2)	23.1 (22–24)	14.6 (14–16)	4.8 (4.8–5.0)	0.46	21.3	33.8	721 (600–946)
sigmaturellus	U.S.A. (Calif.)	3 ♀♀ 1 ♂	2.3 (1.6–2.6)	34.5 (30–39)	18.7 (17–20)	6.4 (6.0–7.0)	0.29	18.7	34.2	651 (450–836)
prodenticulatus	Australia	1 ♂	2.2	33	18	7.5	0.34	23	42	966
index	U.S.A. (Calif.)	3 ♀♀, 1 ♂	1.2 (1.1–1.4)	21 (20–22)	11	5.4 (5.0–6.0)	0.45	26.0	50	1305 (1125–1485)
index	Hawaii	2 ♀♀	0.6–0.8	18	8	3.1–4.0	0.50	20	45	663–1100

genus *Mylonchulus* vary from 0.61 mm (*M. index*) to 2.90 mm (*M. sigmaturellus* n. sp.). Tail shape and length vary widely between species but are fairly constant within species. These characters are used extensively in species separation. Position of the spinneret opening is generally constant within species of *Mylonchulus*. Caudal glands, when present, are either grouped or in tandem, and this arrangement is sometimes used in the separation of some species.

Amphids or lateral organs are located on the lateral lines somewhere between the base of the labia and a point opposite the middle of the buccal cavity. The amphid aperture is generally distinct in this genus and therefore width of the aperture and position of the amphid was studied to determine extent of differences within and between species. Amphid aperture width was determined from camera lucida drawings.

Since there appears to be a wide variation in the position of the amphid aperture (Table I) within each species, this character would be of little value in the separation of species.

Amphid aperture width is fairly constant in most species examined (Table II). Differences in amphid aperture width might be used in separating *M. brevicaudatus* from *M. incurvus*. *M. montanus*, although its buccal cavity measurements are nearly the same as those of *M. incurvus*, has an amphid aperture width range which does not reach the lowest figure for that of *M. incurvus*. Therefore, amphid aperture width and its relationship with buccal cavity size could be considered in the separation of species in the genus *Mylonchulus*.

Taxonomy

The genus *Mononchus* was erected by Bastian (7) in 1865. Cobb (13) proposed five subgenera within this genus. These were: *Mononchus*, *Mylonchulus*, *Prionchulus*, *Iotonchus*, and *Anatonchus*. In 1917 Cobb used these subgenera in his monograph (14) of the Mononchidae. He also set up a new subgenus, *Sporonchulus*. Micoletzky (34) also considered this family in detail. Andrássy (4), who brought together the work of Cobb and Micoletzky, raised all the subgenera of Cobb to generic rank and erected five new genera. The family Mononchidae (Chitwood and Chitwood, 1937), according to Andrássy (4), now consists of 11 genera. These are: *Anatonchus*, *Brachonchulus*, *Cobbonchus*, *Granonchulus*, *Iotonchus*, *Judonchulus*, *Miconchus*, *Mononchus*, *Mylonchulus*, *Prionchulus*, and *Sporonchulus*.

Andrássy (4) made a valuable contribution in his reclassification of this group and in revising the work of Micoletzky (34). However, most of Andrássy's work deals with the genera, and the individual species, with some exceptions (5), are not considered in detail. The need for further work in this connection was realized and therefore I am undertaking, in a series of papers, to fill such gaps in this family. The present paper deals with the genus *Mylonchulus*. The classification of Andrássy (4) is followed generally.

Genus *Mylonchulus* (Cobb, 1916) Altherr, 1953 (and Andrássy, 1958)

Definition.—Dorsal tooth large to massive, situated midway or forward in goblet-shaped buccal cavity. Two small subventral teeth opposing dorsal

tooth present or absent. Series of small denticles present on two subventral sectors of buccal cavity forming rasp-like areas. Gonads paired or unpaired. Tail mostly short with subterminal or terminal spinneret present in most species. Caudal glands present or absent.

Type species.—*Mylonchulus minor* (Cobb, 1893) Andrássy, 1958.

Key to Species of Genus *Mylonchulus*

Females

1. Ovary 1 2
   Ovaries 2 4
2. Ovary posterior to vulva ..... *reversus* (Cobb)
   Ovary anterior to vulva 3
3. Tail conoid truncate, spinneret opening dorsal ..... *subterraneus* (Schneider)
   Tail irregular, spinneret opening terminal ..... *index* (Cobb)
4. Spinneret absent or rudimentary 5
   Spinneret present 8
5. Tail rounded, spinneret rudimentary 6
   Tail not rounded, spinneret absent 7
6. Rudimentary spinneret opening dorsal, tail short ..... *striatus* (Thorne)
   Rudimentary spinneret opening ventral, tail longer ..... *rotundicaudatus* (Skwarra)
7. Tail conoid, becoming cylindroid in posterior half ..... *solus* n. sp.
   Tail conoid arcuate ..... *subsimilis* (Cobb)
8. Spinneret opening terminal 9
   Spinneret opening subterminal 19
9. Tail sharply bent near middle or slightly posterior to level of anus 10
   Tail arcuate, conoid, or clavate 13
10. Tail sharply bent near middle, dorsal curvature smooth and even ..... *incurvus* (Cobb)
    Tail sharply bent near middle or slightly posterior to level of anus, dorsal curvature flattened or uneven posteriorly 11
11. Tail conoid then strikingly cylindroid just posterior to level of anus. *cavensis* (Schneider)
    Tail conoid then strikingly cylindroid in posterior half or less 12
12. Labia prominently flared, cylindroid part of tail more than one-half total tail length ..... *sigmaturellus* n. sp.
    Labia not prominently flared, cylindroid part of tail about one-third total tail length ..... *sigmaturus* (Cobb)
13. Tail clavate, dorsally recurved ..... *clavicaudatus* (Schuurmans Stekhoven & Teunissen)
    Tail acuate conoid, not dorsally recurved 14
14. Dorsal tooth small, somewhat digitate, anterior refractive ring very prominent ..... *obtusicaudatus* (Daday)
    Dorsal tooth relatively massive, anterior refractive ring much less prominent 15
15. Buccal cavity about as wide as long, submedian teeth very minute ..... *minor* (Cobb)
    Buccal cavity much longer than wide, submedian teeth relatively large and distinct 16
16. Buccal cavity large (av. 34×21 microns), walls prominently striated, caudal glands always distinct and in tandem ..... *montanus* (Thorne)
    Buccal cavity medium-sized, walls obscurely striated, caudal glands obscure to distinct 17
17. Tail short (c = 40–47), strongly arcuate ..... *sexcristatus* (Merzheevskaya)
    Tail medium length (c = 33.0 or less), uniformly conoid 18
18. Anterior lip of anus massive, overhanging, V = 72% ..... *subtenuis* (Cobb)
    Anterior lip of anus not massive, V = 60% (approx.) ..... *lacustris* (Cobb, in Cobb)
19. Spinneret dorsally recurved, dorsal tooth massive, walls of buccal cavity prominently striated ..... *brevicaudatus* (Cobb)
    Spinneret not dorsally recurved, dorsal tooth small to large, buccal cavity walls faintly striated or none 20
20. Tail very short (c = 50), terminus blunt, truncate ..... *obliquus* (Cobb)
    Tail longer, terminus more acute 21
21. Tail long (c = 25), caudal glands in tandem, denticles relatively large ..... *parabrachyurus* (Thorne)
    Tail shorter (c = 30), caudal glands generally grouped, denticles relatively small, numerous ..... *brachyuris* (Buetschli)

KEY TO SPECIES OF GENUS *Mylonchulus*

MALES

1. Denticles mostly anterior to level of dorsal tooth apex, submedian teeth large, nearly basal ..... *prodenticulatus* n. sp.
   Denticles posterior to level of dorsal tooth apex, submedian teeth about midway in buccal cavity 2
2. Spinneret absent or rudimentary 3
   Spinneret present 5
3. Spinneret absent, tail conoid arcuate ..... *subsimilis* (Cobb)
   Spinneret rudimentary, tail rounded or hemispherical 4
4. Spinneret opening dorsal, tail short, terminus rounded, spicule short (37 microns) ..... *striatus* (Thorne)
   Spinneret opening ventral, tail longer, terminus hemispherical, spicule long (65 microns) ..... *rotundicaudatus* (Skwarra)
5. Spinneret opening terminal 6
   Spinneret opening subterminal 11
6. Tail uniformly conoid, caudal glands in tandem, accessory pieces obscure ..... *montanus* (Thorne)
   Tail irregular, caudal glands generally grouped, accessory pieces bifurcated or not bifurcated 7
7. Tail sharply bent ventrally near middle, not cylindroid in posterior part ..... *incurvus* (Cobb)
   Tail sharply bent near middle or slightly posterior to level of anus, cylindroid in posterior part 8
8. Tail presenting effect of closed fist with pointing index finger, amphid aperture relatively wide ..... *index* (Cobb)
   Tail not as above, amphid aperture relatively shorter 9
9. Tail conoid, then strikingly cylindroid just posterior to level of anus. *cavensis* (Schneider)
   Tail conoid, then strikingly cylindroid in posterior half or less of tail 10
10. Labia prominently flared, cylindroid part of tail at least one-half total tail length ..... *sigmaturellus* n. sp.
    Labia not prominently flared, cylindroid part of tail about one-third total tail length ..... *sigmaturus* (Cobb)
11. Spinneret dorsally recurved, dorsal tooth massive, walls of buccal cavity prominently striated ..... *brevicaudatus* (Cobb)
    Spinneret not dorsally recurved, dorsal tooth small to large, walls of buccal cavity sometimes faintly striated 12
12. Tail long ($c = 25$), caudal glands in tandem, denticles relatively large ..... *parabrachyurus* (Thorne)
    Tail shorter ($c = 32$), caudal glands generally grouped, denticles relatively small, numerous ..... *brachyuris* (Buetschli)

Mylonchulus brachyuris (BUETSCHLI, 1873) ALTHERR, 1953
(Figs. 3–6)

Canadian Specimens

(*10 females*).—$L = 1.23$ mm (1.13–1.38); $a = 25.5$ (20.0–30.2); $b = 3.5$ (3.1–3.6); $c = 28.8$ (25.1–34.5); $V = 61\%$ (57–64); buccal cavity 21–23 × 12–16 μ; tail length = 0.043 mm (0.040–0.046).

(*5 males*).—$L = 1.22$ mm (1.03–1.40); $a = 29.6$ (27.0–32.5); $b = 3.5$ (3.3–3.7); $c = 32.1$ (27.1–36.3); spicule length = 47.6 μ (40–55); buccal cavity = 20–23 × 12–17 μ; tail length = 0.036 mm (0.030–0.038); supplements = 12.

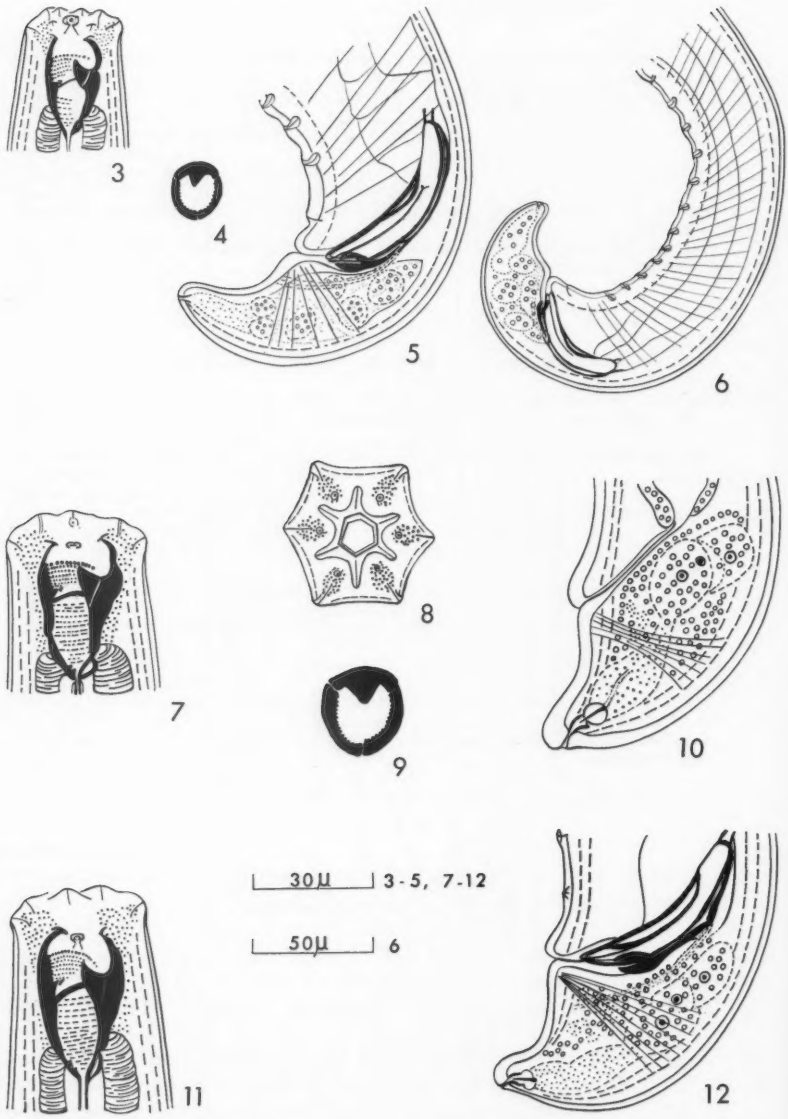
The Canadian specimens fit Cobb's (14) description of this species fairly well in details of anterior part of body and tail. However, in most of the Canadian specimens the walls of the buccal cavity are faintly striated.

Five males were found in a population of several hundred females. Details of the buccal cavity were the same as those of the female. Caudal glands in the male tail were obscure but otherwise details of the tail were the same as those of the female *brachyuris*. de Man (24), in his description of the first male

TABLE III

M. brachyuris from foreign countries

Locality	No. of specimens	Length of body (av.) (mm)	*a*	*b*	*c*	*V* (%)	Tail length (mm)	Buccal cavity (μ)
Holland	4 ♀♀	1.09 (0.97–1.36)	25.0 (23.4–28.5)	3.9	36.0 (34.0–37.3)	59	0.035 (.026–.040)	20–23×12–13
Holland	1 ♂	1.15	30.3	3.6	35.9	—	0.032	21×13
Germany	3 ♀♀	1.17 (1.07–1.25)	27.8 (25.5–29.0)	3.7 (3.6–3.8)	36.5 (32.4–42.5)	60	0.032 (.028–.036)	21–22×13
France	3 ♀♀	1.15 (1.10–1.20)	26.2 (24.4–28.2)	3.8 (3.8–3.8)	38.9 (36.0–40.7)	62	0.029 (.027–.032)	21–22×12
England	1 ♀	1.06	28.6	3.7	29.4	61	0.036	22×13
U.S.A. (Florida)	10 ♀♀	0.92 (0.82–0.97)	24.2 (22.8–26.3)	3.4 (3.2–3.8)	28.5 (23.4–32.0)	57	0.033 (.027–.042)	20–21×11–13
Venezuela	6 ♀♀	0.86 (0.67–1.08)	22.9 (21.8–25.1)	3.4 (3.2–3.7)	24.8 (23.2–27.0)	60	0.035 (.027–.042)	18–20×10



FIGS. 3–6. *Mylonchulus brachyuris*. 3. Male head. 4. *En face* view of buccal cavity. 5. Male tail. 6. Male tail showing arrangement of supplements.

FIGS. 7–12. *Mylonchulus brevicaudatus*. 7. Female head. 8. *En face* view of labia and papillae. 9. *En face* view of buccal cavity. 10. Female tail. 11. Male head. 12. Male tail showing spicules and gubernaculum.

found for this species, describes two bifurcated accessory pieces, and the spicules (according to his illustration) measure approximately 65 μ in length. His illustration shows no gland or spinneret in the tail. de Man's males are probably *M. sigmaturus*. Goodey (19) also illustrated bifurcated accessory pieces in his illustration of the male *brachyuris* from England while the spicule of this male measured 50 microns in length. These males may also be *M. sigmaturus*. Thorne (46) did not find bifurcated accessory pieces in a closely related species, *M. parabrachyurus*. After careful examination of several male *brachyuris* from Canada and one male from Holland, I found no bifurcated accessory pieces. I have also examined female specimens of *M. brachyuris* from Holland, Germany, France, England, Venezuela, and Florida, U.S.A. The measurements of these specimens are contained in Table III.

The specimens from Holland, Germany, and France fitted Cobb's description very well. Specimens from Florida, Venezuela, and England had relatively longer tails than those from the other countries. Those from Florida had three caudal glands in tandem leading into a well-developed subterminal spinneret. Denticles in the buccal cavities of all specimens were relatively the same size.

M. brachyuris, which was first described and illustrated by Buetschli (8), has since been recorded or studied by many workers including de Man (25), Marcinowski (27), Ditlevsen (16, 17, 18), Hoffmänner and Menzel (23), Seidenschwarz (42), Micoletzky (35), Allgén (1), Hoeppli (22), Altherr (3), and Goodey (20). It is a cosmopolitan species.

Mylonchulus brevicaudatus (Cobb, 1917) Altherr, 1954
(Figs. 7–12)

Canadian Specimens

(*10 females*).—L = 1.54 mm (1.38–1.70); a = 26.0 (24.2–30.0); b = 3.1 (2.9–3.2); c = 44.7 (41.0–58.0); V = 65% (63–67); buccal cavity = 30–36 × 17–20 μ; tail length = 0.035 mm (0.028–0.040).

(*3 males*).—L = 1.56 mm (1.40–1.68); a = 29.0 (25.9–31.7); b = 3.1 (2.9–3.2); c = 40.8 (40.0–42.0); buccal cavity = 32–35 × 16–19 μ; spicule length = 52.3 μ (50–55 μ); gubernaculum length = 23.6 μ (24–25 μ); tail length = 0.038 mm (0.035–0.040); supplements = 14–15.

The females conform to Cobb's description (14) very well. Dorsal tooth massive, submedian teeth small and inconspicuous, amphids narrow and conspicuous. Buccal cavity large with thick, prominently striated walls. Denticles medium-sized in 6 or 7 rows. Posterior refractive ring distinct. Intestine prominently tesselated with large nucleated cells. Ovaries two, reflexed one-half distance to vulva, uterus containing one or two eggs averaging 110×50 μ in size. Sperm found in reproductive tract of several of the many females examined. Tail same as that described by Cobb (14). Spinneret dorsal with needle-shaped valve. Caudal glands three, oval-shaped and grouped.

Male.—Details of the head and the tail are the same as for the female. This is the first record of the male of this species. Testes double, reflexed slightly, well supplied with sperm. Spicules short, with pointed distal ends. Gubernaculum thick, bifid, and about one-half length of spicule.

TABLE IV

Comparative measurements of *Mylonchulus brachyuris*, *M. minor*, and *M. brevicaudatus*

Species	Body length (mm)	Buccal cavity			Dorsal tooth	Tail length	*c*-value
		L (μ)	*W* (μ)	Striae			
M. brachyuris	1.2–2.0	20	14	Obscure	Small?	0.048–0.080	25.0
M. minor	1.1	25	24	None	Small	0.036	30.3
M. brevicaudatus	1.5	32	20	Prominent	Massive	0.030	50.0

Cobb (14) gave an adequate description of the female of this species. He remarked that *M. brevicaudatus* resembled *M. brachyuris* and *M. minor* in general form but differed in details of the pharynx and the tail. The three species are compared in Table IV (all measurements after Cobb, 1917).

Andrássy (4) made *M. brevicaudatus* a synonym of *M. micrurus* on the basis of similar stomal and tail structures. Cobb (14) claimed that *M. micrurus* resembled *M. brevicaudatus*. I found, after carefully examining a number of second-stage juveniles of *M. brevicaudatus*, that the two submedian teeth, which, according to Cobb (14), are absent in *M. micrurus*, are very small and in some specimens very difficult to find. A similar condition exists in most of the mature females. Consequently I consider *M. brevicaudatus* a valid species. Measurements of four specimens were as follows:

Second-stage juveniles.—L = 1.15 mm (1.00–1.32); a = 33.0 (28.1–37.6); c = 45.8 (40.0–49.6); buccal cavity = 25–28 × 12–14 μ; tail length = 0.025 mm (0.022–0.033).

Habitat.—About roots of cranberry in cranberry bog, and in sphagnum moss.

Geographical distribution.—New Jersey, U.S.A.; Capri, Italy; Ottawa, Canada.

Mylonchulus cavensis (Schneider, 1940) Andrássy, 1958

(*Female*).—L = 1.32 mm; a = 30; b = 3.5; c = 26.5; V = 66%; tail = 0.045 mm.

(*Male*).—L = 0.806 mm; a = 32; b = 3.4; c = 21.5; spicule length = 32 μ; supplements = 10.

This species was erected on the basis of one male and four females. Schneider (38) gave an adequate description of the female but for the male he remarked only that the spicules were bulky and that there were 10 preanal papillae. The details and structure of the tail distinguish this species from all others. Schneider's only illustration is that of the female tail.

Habitat.—Aquatic.

Geographical distribution.—Ljubljana, Jugoslavia; Italy.

Mylonchulus clavicaudatus (Schuurmans Stekhoven & Teunissen, 1938) Andrássy, 1958

(*1 female*).—L = 0.94 mm; a = 27; b = 3.9; c = 36.3; V = 66%; tail length = 0.026 mm.

Species erected on one female. Adequate description and illustrations

are presented by Schuurmans Stekhoven and Teunissen (41). This species has a toothed rib extending nearly the full length of the buccal cavity. The amphids are nearly basal. The authors (41) concluded that this species is a combination of *Prionchulus* and *Mylonchulus*. The clavate tail and the details of the pharynx distinguish this species from others in this genus.

Habitat.—Soil.

Geographical distribution.—Rutshuru, Belgian Congo.

Mylonchulus incurvus (Cobb, 1917) Andrássy, 1958
(Figs. 13–19)
Syn. *M. hawaiiensis* Cassidy, 1931

Canadian Specimens

(*3 females*).—L = 1.82 mm (1.62–2.00); a = 27.0 (24.0–30.8); b = 3.1 (2.8–3.2); c = 39.8 (37.0–47.5); V = 65% (63–67); buccal cavity = 36–38 × 21–23 μ; tail length = 0.047 mm (0.040–0.052).

California Specimens

(*3 females*).— L = 2.14 mm (2.02–2.33); a = 33.3 (31.8–34.8); b = 3.6 (3.3–3.8); c = 40.4 (38.1–44.0); V = 65% (63–68); buccal cavity = 35–40 × 21–23 μ; tail length = 0.053 mm.

(*3 males*).—L = 2.16 mm (1.90–2.48); a = 39.3 (37.0–43.8); b = 3.4 (3.1–3.7); c = 39.1 (35.0–44.3); spicule length = 69.3 μ (68–72); buccal cavity = 34–36 × 19–20 μ; tail length = 0.054 mm (0.050–0.060); supplements = 14–15.

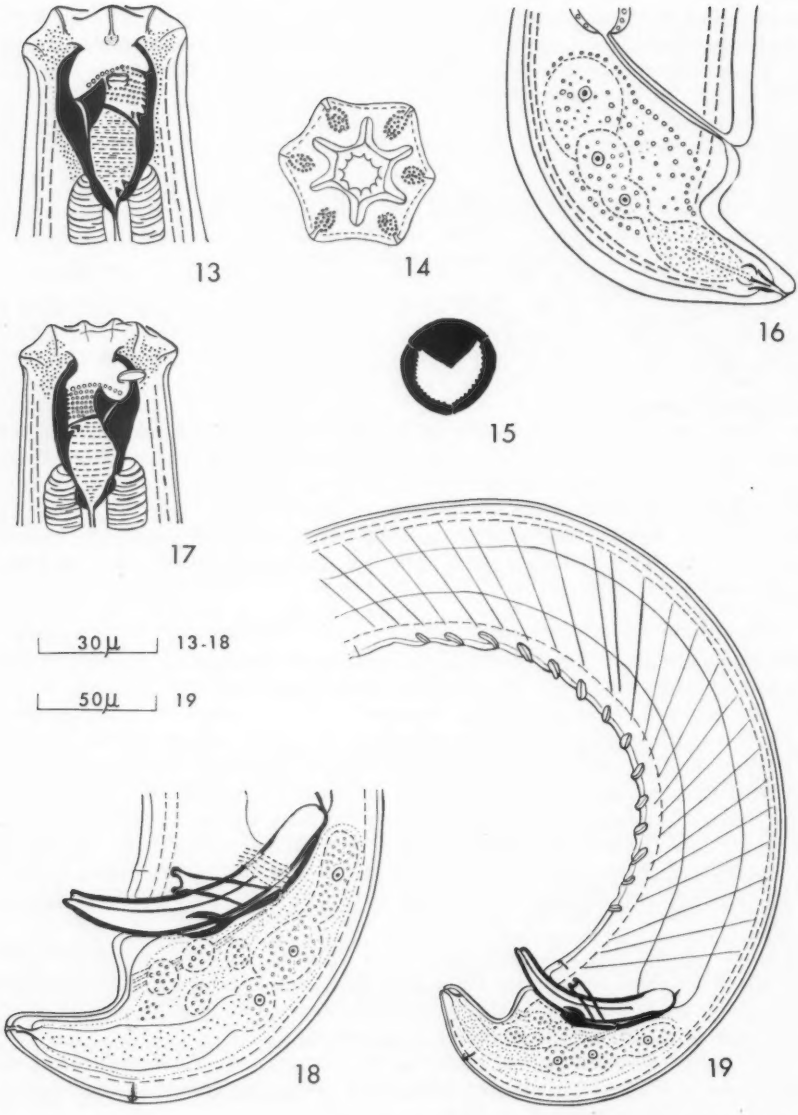
Description of females from Canada and California as follows: Labia prominent, papillae distinct, dorsal tooth massive, anterior. Amphid aperture relatively wide, buccal cavity about twice as long as wide, walls thick, prominently striated. Denticles large, arranged in 6 or 7 rows. Refractive rings anterior and posterior to denticles. Anterior ring thin and sometimes difficult to observe. Submedian teeth relatively large. Intestine partly tesselated, ovaries two, reflexed one-half distance to vulva, spermatheca of California specimens containing many sperm. Valve between uterus and spermatheca. Tail arcuate, heavily muscled, sharply bent about midway to terminus. Caudal glands three, in tandem, saccate, emptying into a large ampulla leading to a slightly subterminal armed spinneret equipped with a needle-shaped valve.

Male.—Head and tail details same as those of female. This is the first record of a male of this species. Gonads double, slightly reflexed, and containing many sperm. Spicules long, slender, with bifurcated accessory pieces. Gubernaculum thick, about one-third length of spicule.

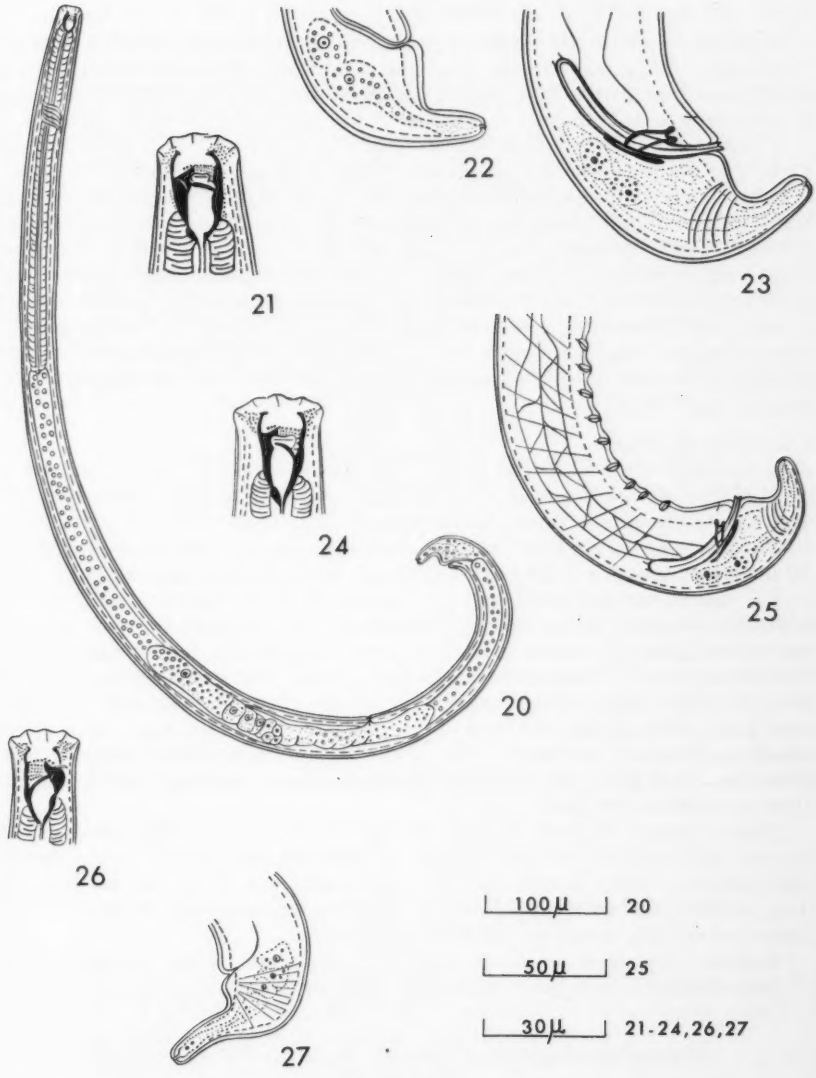
Andrássy (4) considers *M. incurvus* a synonym of *M. sigmaturus*. I do not agree since there are distinct differences in the details of the buccal cavity and tail between these two species.

Habitat.—Bog soil; sandy soil in cranberry bog.

Geographical distribution.—California and Virginia, U.S.A.; Jamaica; Mauritius; Ontario, Canada.



FIGS. 13–19. *Mylonchulus incurvus*. 13. Female head. 14. *En face* view of labia and papillae. 15. *En face* view of buccal cavity. 16. Female tail. 17. Male head. 18. Male tail showing spicules and bifurcated accessory piece. 19. Male tail showing arrangement of supplements.



FIGS. 20–25. *Mylonchulus index* (specimens from U.S.A.). 20. Female. 21. Female head. 22. Female tail. 23. Male tail showing spicules and bifurcated accessory piece. 24. Male head. 25. Male tail showing arrangement of supplements.

FIGS. 26, 27. *Mylonchulus index* (specimen from Hawaii). 26. Female head. 27. Female tail.

Mylonchus index (COBB, 1906) ANDRÁSSY, 1958
(Figs. 20–27)

Cobb (12) gave an adequate description of this species but did not include illustrations. Williams (48) described and illustrated specimens of this species which were taken from Mauritian cane fields. The following is a description of specimens from Hawaii:

(*2 females*).—L = 0.610, 0.835; a = 27.7, 32.0; b = 2.7, 3.2; c = 20.3, 27.0; V = 69, 72%; buccal cavity = 18 × 8 μ; tail length = 0.030, 0.031 mm.

Labia fairly prominently flared, buccal cavity slightly longer than wide, walls relatively thin. Posterior refractive ring conspicuous, dorsal tooth relatively large, submedian teeth absent. Amphid aperture slit-like, denticles in six rows. Intestine composed of large nucleated cells, ovary single, extending anterior and reflexed one-half distance to continuous vulva. Tail bulky in anterior half, decreasing suddenly to slender in posterior half. Caudal glands three, grouped, emptying into a terminal spinneret. These specimens conform to Cobb's description of this species fairly well. Only two females and one juvenile were found.

California Specimens

(*4 females*).—L = 1.22 mm (1.07–1.40); a = 34.3 (29.7–46.6); b = 3.4 (3.1–3.5); c = 36.8 (32.4–46.6); V = 76% (75–78); buccal cavity = 20–22 × 10–11 μ; tail length = 0.033 mm (0.030–0.040).

(*1 male*).—L = 1.119 mm; a = 35.0; b = 3.4; c = 25.8; spicule length = 50 μ; buccal cavity = 22×11 μ; tail length = 0.046 mm; supplements = 9.

The specimens from California fit Cobb's description for this species very well. Dorsal tooth unopposed by submedian teeth. Reflexed anterior ovary well developed; one female contained a thin-shelled egg (70×36 μ in size). Sperm was found in the spermatheca of one female. The rudimentary posterior branch of the reproductive tract extends to about one and one-half times the anal body width behind the level of the vulva opening. There are two or three small papillae just anterior to the vulva. The tail is the same shape as that described by Cobb (12) and contains three rather obscure caudal glands. The spinneret is terminal.

Male.—Details of head and tail same as those of female. Amphid aperture is very wide, just below apex of tooth. This is the first record of the male of this species. Testes double, outstretched, containing many sperm. Spicules long, slender, and with turned-out distal ends. Gubernaculum about one-third length of spicule, accessory pieces two, bifurcated.

Habitat.—Soil from apple orchard (U.S.A.), about roots of sugar cane.

Geographical distribution.—Mauritius, Hawaii, and California.

Mylonchulus lacustris (COBB, M. V., 1915) ANDRÁSSY, 1958
(Figs. 28–29)

The original description of this species (10) did not include illustrations; Cobb (14) redescribed and illustrated the head and tail. He remarked that the amphids had the form of slits, and that the ventrally arcuate tail contained three caudal glands arranged in tandem and ended in a blunt spinneret.

He concluded that this species resembled *M. brachyuris* and *M. polonicus*. Andrássy (4) considers *M. lacustris* a synonym of *M. obstusicaudatus*. I do not agree with this synonymy because of the differences in details of the buccal cavity and tail shape.

The following measurements were made of specimens from Florida and Missouri, U.S.A.:

(*5 females*).—L = 1.09 mm (1.00–1.20); a = 27.3 (22.2–33.3); b = 3.9 (3.6–4.3); c = 23.2 (19.2–26.2); V = 55% (52–58); buccal cavity = 20–23 ×12–14 μ; tail length = 0.047 mm (0.042–0.052).

Walls of buccal cavity distinctly striated, posterior refractive ring distinct. Dorsal tooth relatively large opposed by two distinct submedian teeth. Denticles fairly large, in 5 or 6 rows. Intestine distinctly tesselated. Ovaries two, reflexed, uterus containing thin-shelled eggs. Tail ventrally arcuate, caudal glands three, in tandem, leading into a terminal spinneret.

Habitat.—Garden soil.

Geographical distribution.—United States, Panama Canal Zone, Europe, Japan, South Africa, Mauritius, Australia.

Mylonchulus minor (Cobb, 1893) Andrássy, 1958

(*Female*).—L = 1.1 mm; a = 29.4; b = 3.8; c = 30.3; V = 60%; buccal cavity = 25×24 μ; tail length = 0.036 mm.

Cobb (11) based his description and illustrations on an immature female. He remarked that the head of this species was less truncate than usual and in his key he stated that the pharynx was about as long as wide. Cobb (14) redescribed this species, emphasizing the presence of two minute submedian teeth. He remarked that the conoid tail was rather strongly arcuate and bent somewhat near the middle. His illustration showed that the walls of the buccal cavity are striated. The buccal cavity measures about 25×24 μ. The shape and size of the buccal cavity and the minute submedian teeth separate this species from closely related species such as *M. lacustris*. Schuurmans Stekhoven (40) described and illustrated a doubtful specimen of this species.

Habitat.—Soil.

Geographical distribution.—Fiji.

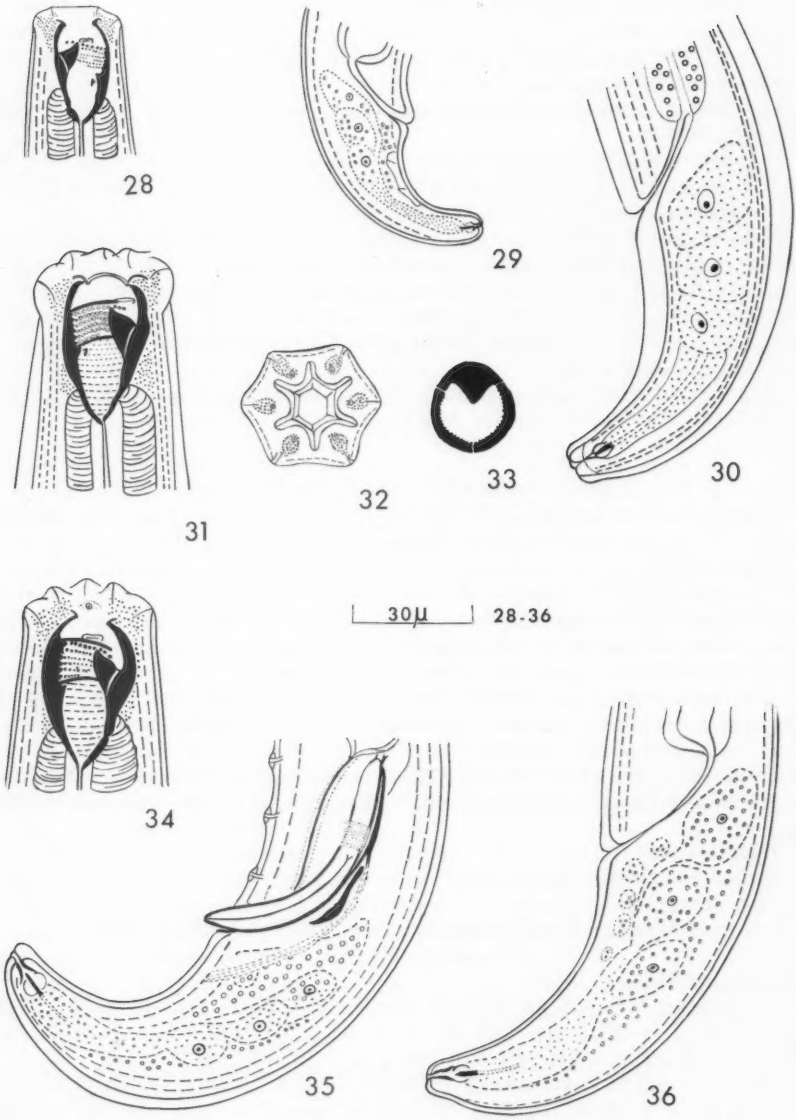
Mylonchulus montanus (Thorne, 1924) Andrássy, 1958
(Figs. 30–36)

Canadian Specimens

(*10 females*).—L = 1.82 (1.60–1.90 mm); a = 33.1 (26.4–37.2); b = 3.5 (3.2–3.6); c = 20.1 (16.5–24.0); V = 62.5% (59–66); buccal cavity = 33–35 × 20–22 μ; tail length = 0.092 mm (0.075–0.105).

(*3 males*).—L = 1.65 mm (1.55–1.80); a = 30.2 (27.3–32.0); b = 3.2 (3.1–3.3); c = 20.0 (18.2–21.1); spicule length = 64 μ (60-68); buccal cavity = 33–39 × 20–21 μ; tail length = 0.082 mm (0.080–0.085); supplements = 13–14.

The females in the Canadian collection fit Thorne's description (46) well despite the existence of minor differences in the size of the buccal cavity.



FIGS. 28, 29. *Mylonchulus lacustris*. 28. Female head. 29. Female tail.
FIGS. 30–36. *Mylonchulus montanus*. 30. Female (young) tail. 31. Female head. 32. *En face* view of labia and papillae. 33. *En face* view of buccal cavity. 34. Male head. 35. Male tail showing spicules and gubernaculum. 36. Female (mature) tail.

Dorsal tooth massive opposed by two relatively large submedian teeth. Denticles large, in 6 rows with distinctive anterior and posterior refractive rings. Buccal cavity large, walls thick and prominently striated. Tail conoid arcuate, three caudal glands in tandem emptying into a large ampulla which leads to a terminal spinneret equipped with a needle-shaped valve. Constriction on ventral side of tail terminus distinct in some specimens. This species is common in Canada.

The male of this species is herein described for the first time. Three males were found among at least two hundred females. Details of buccal cavity and tail same as those of the female. Testes double, amply supplied with sperm. Spicules long, stout, and provided with a gubernaculum about one-third their length. Preanal supplements slightly raised above level of cuticle.

Thorne (46) in his description of this species stated that it resembled *M. subtenuis* and might possibly be identical with *M. polonicus*. The three species are compared in Table V.

M. montanus resembles *M. polonicus* in details of the tail shape and structure. However, because of inadequate illustration by Stefanski (44), comparison of the details of the buccal cavities of these two species is difficult. For this reason I disagree with Andrássy's (4) synonymy of *M. montanus* with *M. polonicus*.

Habitat.—Bog soil.

Geographical distribution.—Utah, U.S.A.; Ottawa, Canada.

TABLE V

Comparative measurements of *Mylonchulus polonicus*, *M. subtenuis*, and *M. montanus*

Species	Body length (mm)	Tail length (mm)	Buccal cavity (μ)	*c*-value
M. polonicus	1.43	0.068	?	21.0
M. subtenuis	1.9	0.057	28×18	33.3
M. montanus	1.8–2.5	0.084–0.117	37×29	21.3

Mylonchulus obliquus (Cobb, 1917) Andrássy, 1958

Syn. *Mylonchulus japonicus* (Cobb, 1917) Andrássy, 1958

(*Female*).—L = 1.4 mm; a = 24.4; b = 3.2; c = 50.0; V = 63%; buccal cavity = 20×13 μ; tail length = 0.028 mm.

Cobb (14) described and illustrated this species. He described but did not illustrate a closely related species, *M. japonicus*, which I agree with Andrássy (4) is a synonym of *M. obliquus* because of close similarity in the details of head and tail and relative body measurements. Cobb stated that *M. japonicus* had a short, blunt arcuate tail which was truncated at the terminus. The *c*-value for both species is 50.

Habitat.—Soil.

Geographical distribution.—Germany.

Mylonchulus obtusicaudatus (Daday, 1899) Andrássy, 1958

(*Female*).—L = 1.9 mm; a = 21.3; b = 3.6; c = 21.3; V = 67%; buccal cavity = 40×30 μ; tail length = 0.089 mm.

Daday's illustration (15) shows a rather wide buccal cavity with thick walls and a small dorsal tooth with two smaller submedian teeth. The anterior refractive ring about the buccal cavity is very prominent. The rather long tail ($c = 21.3$) is conoid arcuate and contains several glands which empty through a terminal spinneret.

Cobb (14) considered that this species is identical with *M. minor*. However, the prominent anterior refractive ring, the small dorsal tooth, and the relative size of the buccal cavity distinguish the two species.

Habitat.—Fresh water.

Geographical distribution.—Berlinhaven Island of Seleo, New Guinea.

Mylonchulus parabrachyurus (THORNE, 1924) ANDRÁSSY, 1958

(*Female*).—$L = 1.5$ mm; $a = 29.4$; $b = 3.8$; $c = 25.0$; $V = 62\%$; buccal cavity = 24×14 μ; tail length = 0.061 mm.

(*Male*).—$L = 1.58$ mm; $a = 33.3$; $b = 3.8$; $c = 25.0$; spicule length = 60 μ; tail length = 0.075; supplements = 10–14.

Thorne (46) gives an adequate description and illustrations of both males and females of this species. He remarks that this species resembles *M. brachyuris* but differs in having a longer tail and caudal glands arranged in tandem. Males of this species are rare and have from 10 to 14 supplementary organs. The tail is similar in form to that of the female. The spicules are strong, arcuate, and with rather slender non-bifurcated accessory pieces.

Habitat.—Light sandy soil.

Geographical distribution.—Utah, U.S.A.

Mylonchulus prodenticulatus N. SP.

(Figs. 37–39)

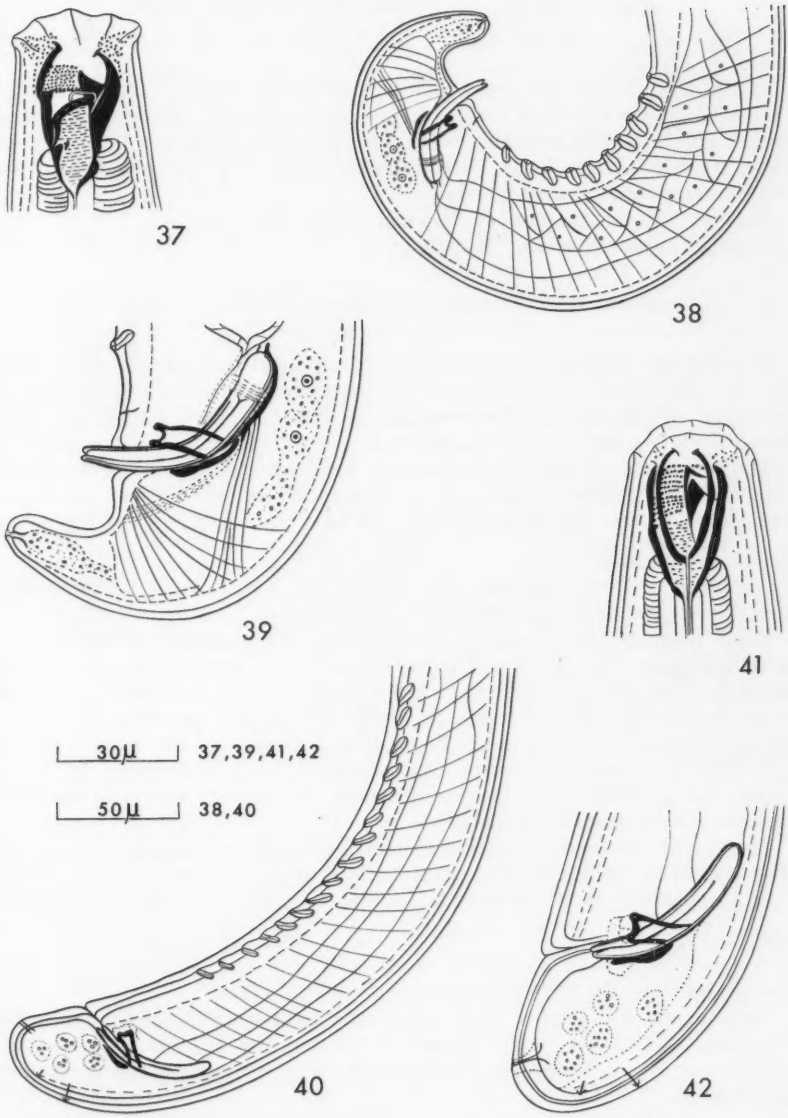
(*Holotype male*).—$L = 2.18$ mm; $a = 44.5$; $b = 3.9$; $c = 42.0$; spicule length = 55 μ; buccal cavity = 33×18 μ; tail length = 0.052 mm; supplements = 10. Collection Australia 16; deposited with the University of California collection at Davis, California.

Labia prominent, buccal cavity large, about half as wide as long, walls thick and prominently striated. Dorsal tooth massive, denticles in 6 or 7 uneven rows immediately anterior to a large even row. Submedian teeth nearly basal, relatively large. Refractive ring conspicuous. Amphid aperture wide and located at base of dorsal tooth. Intestine faintly tesselated. Testes double, containing sperm. Supplementary organs large. Spicule moderately thick, accessory pieces bifurcated. Caudal glands three, rather obscure, tail muscles heavy. Spinneret terminal, ampulla large. Tail short, sharply bent ventrally midway to terminus.

Differential diagnosis.—This species resembles *M. incurvus* in tail structure. *M. prodenticulatus* differs from all other species in the genus by the position of the denticles and submedian teeth, and by the strongly developed refractive ring. Only one male was found.

Habitat.—About roots of grape.

Geographical distribution.—Penrith, Australia.



FIGS. 37–39. *Mylonchulus prodenticulatus* n. sp. 37. Male head. 38. Male tail showing arrangement of supplements. 39. Male tail showing spicule and gubernaculum.
FIGS. 40–42. *Mylonchulus rotundicaudatus* (specimen from U.S.A.). 40. Male tail showing arrangement of supplements. 41. Male head showing last molt of the buccal cavity. 41. Male tail showing spicule and bifurcated accessory piece.

Mylonchulus reversus (COBB, 1917) ANDRÁSSY, 1958

(*Female*).—L = 1.1 mm; a = 29.4; b = 3.8; c = 25.0; V = 40%; buccal cavity = 20×13 μ.

According to Cobb (14) this species resembles *M. incurvus* and *M. minor*. This species differs from all others in the genus in having very few pharyngeal denticles and only one posterior ovary. Buccal cavity small, distinctly striated, dorsal tooth relatively large. Vulva anterior. Cobb (14) based his description and illustrations on gravid females.

Habitat.—About the roots of *Platonia insignis* Mart.

Geographical distribution.—Brazil.

Mylonchulus rotundicaudatus (SKWARRA, 1921) ANDRÁSSY, 1958

(Figs. 40–46)

Skwarra (43) described and illustrated (tail only) this species on the basis of a juvenile female. Allgén (2) gave an adequate description and illustration (tail only) of this species. However, Meyl (31), who fully described and illustrated both the female and male of this species, questioned the validity of Allgén's findings as the latter drew the head area of *Granonchulus schulzi* (Meyl, 1955) Andrássy, 1958, and not that of *M. rotundicaudatus*. The shape and length of the tail separate this species from all others in this genus.

Canadian Specimen

(*1 female*).—L = 1.70 mm; a = 28.3; b = 3.4; c = 34.0; V = 68%; buccal cavity = 35×22 μ; tail length = 0.050 mm.

Head truncate, lips distinct, slit-like amphids located at level of apex of dorsal tooth. Buccal cavity goblet-shaped with very thick, prominently striated walls. Distinct refractive rings forward and posterior to denticles which are small and in 7 rows. Dorsal tooth massive, opposed by two relatively large submedian teeth. Esophagus distinctly glandular in posterior half, intestine tesselated. Ovaries two, reflexed one-half distance to continuous vulva. Tail slightly curved ventrally, terminus bluntly rounded; spinneret ventral, with obscure caudal glands.

The tail of the single Canadian specimen is somewhat longer and narrower than that of *M. rotundicaudatus* described and illustrated by Meyl (31). However, in other details of the head and body there are no marked differences.

Florida Specimen

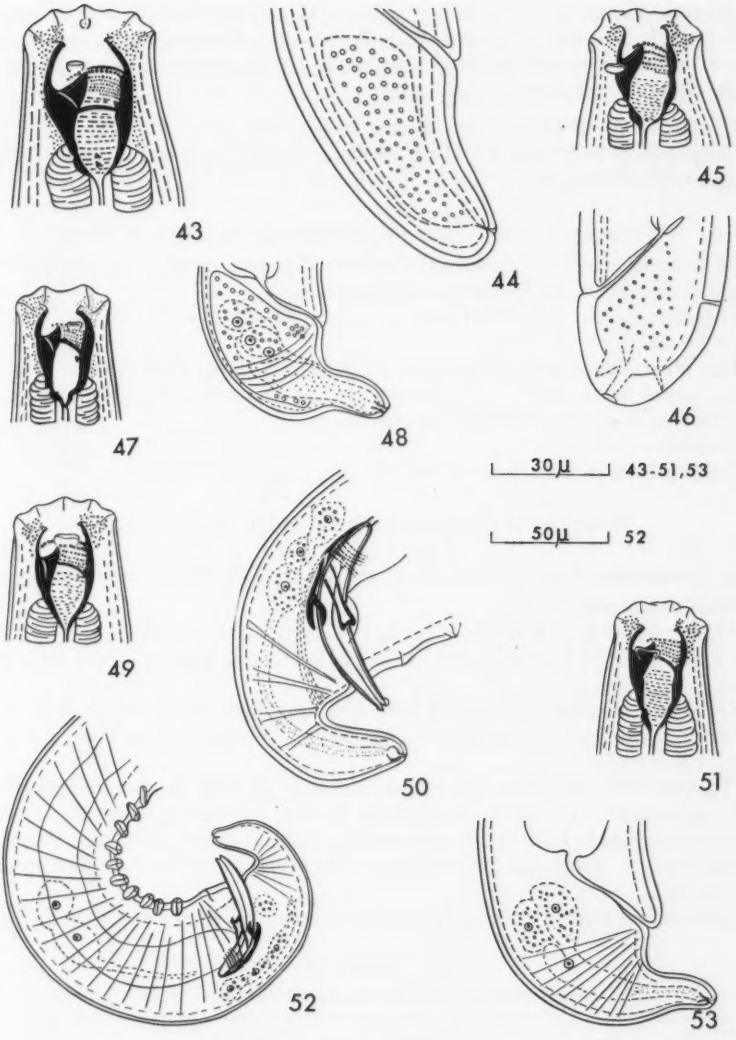
(*1 female*).—L = 1.37 mm; a = 27.9; b = 4.2; c = 48.9; V = 65%; buccal cavity = 25 × 15 μ; tail length = 0.028 mm.

This female was smaller than either the German or Canadian specimens. However, the details of the buccal cavity and the tail fitted *M. rotundicaudatus* very closely. An egg measured 110×40 μ. The spermatheca contained some sperm.

Alabama Specimen

(*1 male*).—L = 1.45 mm; a = 33.7; b = 3.6; c = 45.3; spicule length = 48 μ; buccal cavity = 30×18 μ; tail length = 0.032 mm; supplements = 16.

Details of buccal cavity and tail correspond well with those of the male



FIGS. 43, 44. *Mylonchulus rotundicaudatus* (specimen from Canada). 43. Female head. 44. Female tail.

FIGS. 45, 46. *Mylonchulus rotundicaudatus* (specimen from U.S.A.). 45. Female head. 46. Female tail.

FIGS. 47, 48. *Mylonchulus sigmaturus* (specimen from U.S.A.). 47. Female head. 48. Female tail.

FIGS. 49–53. *Mylonchulus sigmaturus* (specimens from the Netherlands). 49. Male head. 50. Male tail showing spicule and bifurcated accessory piece. 51. Female head. 52. Male tail showing arrangement of supplements. 53. Female tail.

of *M. rotundicaudatus* described by Meyl (31). Testes double, amply supplied with sperm. Accessory pieces with bifurcated distal ends, gubernaculum about one-third spicule length. This male was in the final stage of the last molt. One juvenile female also found.

Habitat.—About roots of sea weeds, swamp soil.

Geographical distribution.— Gulf of Riga, Valga Province, Estonia; Ottawa, Canada; United States.

Mylonchulus sexcristatus (MERZHEEVSKAYA, 1951) N. COMB.

Merzheevskaya (28) apparently erected this species on the basis of several females. She gave the following measurements:

(*? females*).—L = 1.490–1.800 mm; a = 32–40; b = 3.4–4.0; c = 40–47; V = 66–68%.

Her illustrations show that there are at least 5 rows of denticles, a relatively large dorsal tooth, and an unarmed terminal spinneret. She did not include a differential diagnosis for this new species.

Habitat.—?

Geographical distribution.—U.S.S.R.

Mylonchulus sigmaturus (COBB, 1917) ALTHERR, 1953
(Figs. 47–53, 55–58)

Syn. *Mononchus brachyuris* (male of de Man, 1876, female of de Man, 1884)

Canadian Specimens

(*2 females*).—L = 1.45, 1.60 mm; a = 29.0, 32.0; b = 3.5, 3.6; c = 35.6, 39.2; V = 62%; buccal cavity = 25, 27×15 μ; tail length = 0.037, 0.050 mm.

Only two females and several juveniles have been found in Canada. These conform with Cobb's description (14) of this species in details of the buccal cavity and tail.

Thorne (46), who first described the male of this species, remarked that *M. sigmaturus* is instantly recognized by the outlandish ventrally bent tail which is similar in both males and females. Cobb (14) remarked that this species resembled *M. minor* and *M. brachyuris* but differed in the form and structure of the tail.

I examined representatives of this species from several countries. Specimens

TABLE VI

Body measurements of *Mylonchulus sigmaturus* from foreign countries

Locality	No. of specimens	Body length (mm)	Buccal cavity L (μ)	Buccal cavity W (μ)	Tail length (mm)	a	b	c	V (%)
Sicily	2 ♀♀	1.42–1.43	23–25	12	0.038–0.039	34.0	3.4	37.5	63
Italy	5 ♀♀	1.04 (0.95–1.23)	22–24	13–16	0.036 (0.030–0.037)	24.1	3.2	32.6	66 (62–68)
Australia	2 ♀♀	1.10–1.14	22	14	0.030	29.2	3.2	37.3	67

from the United States (California, Nevada, and Florida) were also examined and body measurements and structures compared. Measurements of specimens from Sicily, Italy, and Australia are contained in Table VI.

The three caudal glands in the specimens from Italy were obscure, but were distinct in those from Sicily and Australia, whereas the glands in these specimens were grouped. Thorne (46) reported them as arranged in tandem. I examined 21 females of this species from California and found the caudal

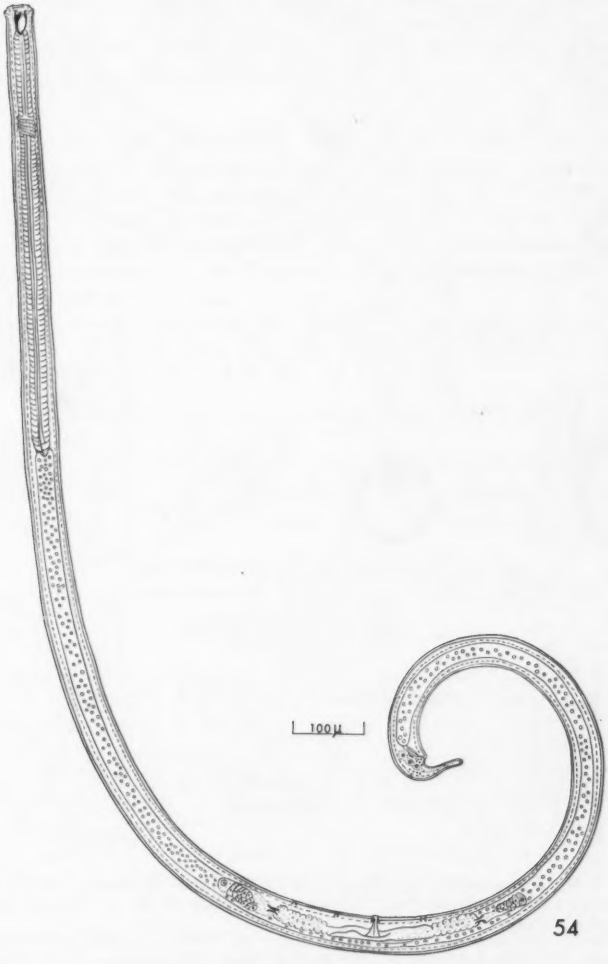
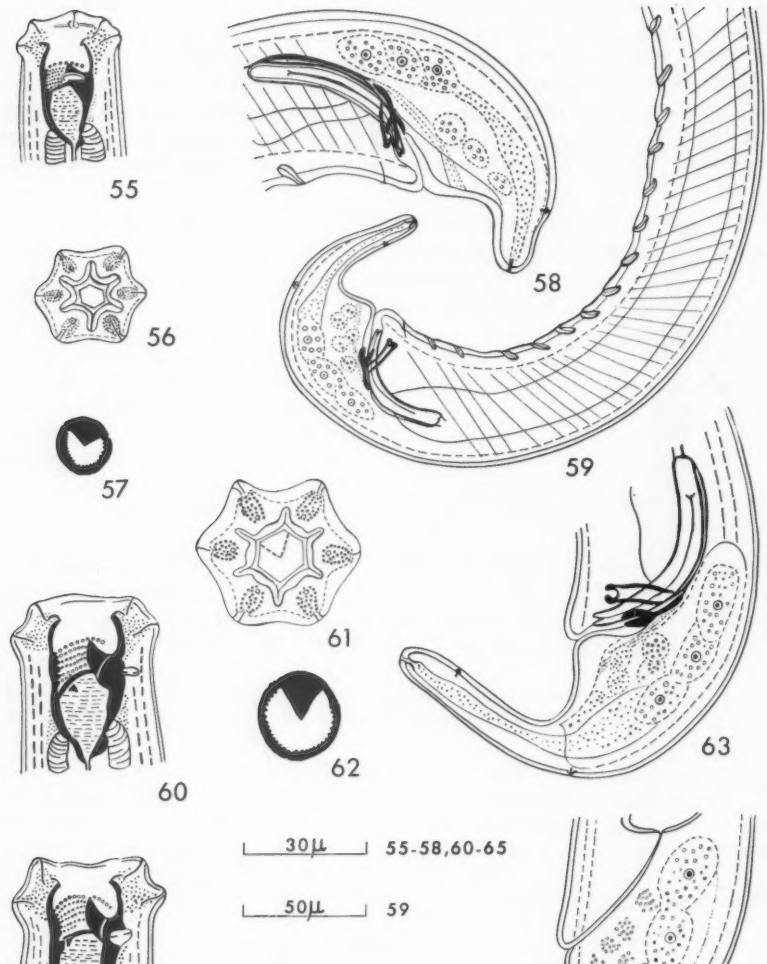


FIG. 54. *Mylonchulus sigmaturellus* n. sp., female.



FIGS. 55–58. *Mylonchulus sigmaturus* (specimens from Germany). 55. Male head. 56. *En face* view of lips and papillae. 57. *En face* view of buccal cavity. 58. Male tail showing spicule and bifurcated accessory piece.

FIGS. 59–65. *Mylonchulus sigmaturellus* n. sp. 59. Male tail showing arrangement of supplements. 60. Male head. 61. *En face* view of labia and papillae. 62. *En face* view of buccal cavity. 63. Male tail showing spicule and bifurcated accessory piece. 64. Female head. 65. Female tail.

glands were grouped in 15 of these, but were obscure in the remaining specimens.

de Man (24, 26) described and illustrated males and females which he identified as *M. brachyuris*. His illustrations indicate that these probably belong to *M. sigmaturus*. I obtained specimens from Germany and Holland for comparison. Both males and females were examined.

Netherlands Specimens

(*2 females*).—L = 1.48, 1.50 mm; a = 29.6, 30.0; b = 3.3, 3.5; c = 39.5, 42.2; V = 61, 65%; buccal cavity = 25×15 μ; tail length = 0.035, 0.038 mm.

(*2 males*).—L = 1.62, 1.83 mm; a = 28.0, 36.6; b = 3.7, 3.8; c = 32.4, 42.6; spicule length = 58, 60 μ; buccal cavity = 23, 25 × 13, 15 μ; tail length = 0.043, 0.050 mm; supplements = 10, 11.

German Specimen

(*1 male*).—L = 1.70 mm; a = 41.4; b = 3.9; c = 41.5; spicule length = 50 μ; buccal cavity = 25×12 μ; tail length = 0.041 mm; supplements = 11.

The male head and tail of the specimens from the Netherlands closely conform to de Man's illustrations (24). The spicules are long with bifurcated accessory pieces. The buccal cavity of the female is striated and the massive dorsal tooth is opposed by two relatively large submedian teeth. The caudal glands are distinct and grouped.

The male German specimen fits Thorne's description (46) in details of head and tail. *M. sigmaturus* has also been either studied or recorded by Schneider (36, 37), and Meyl (29, 30).

Habitat.—Black muck soil, soil in which sugar beets were growing, and about the roots of sugar cane.

Geographical distribution.—Various parts of the United States and Mexico; Oahu, Hawaii; Sicily, Italy; Australia; The Netherlands; Germany; Armstrong, British Columbia, and Moncton, New Brunswick, Canada.

Mylonchulus sigmaturellus N. SP.

Syn. *M. sigmaturoides* (of Schuurmans Stekhoven, 1943 (39) nec Schneider, 1923)

(Figs. 54, 59–65)

(*5 females*).—L = 2.22 mm (1.64–2.65); a = 41.7 (28.2–50.4); b = 3.8 (3.7–4.1); c = 33.8 (31.4–37.8); V = 63% (60–66); buccal cavity = 30–39 ×17–21 μ; tail length = 0.066 mm (0.052–0.073).

(*3 males*).—L = 2.60 mm (2.40–2.90); a = 52.2 (45.3–56.8); b = 4.1 (3.9–4.6); c = 34.8 (31.3–38.0); spicule length = 59.6 μ (56–63 μ); buccal cavity = 29–36 × 15–19 μ; tail length = 0.075 mm (0.063–0.082); supplements = 12–14.

Holotype female.—L = 2.65 mm; a = 47.3; b = 4.1; c = 37.8; V = 60%; buccal cavity = 35×19 μ; tail length = 0.070 mm.

Allotype male.—L = 2.50 mm; a = 54.7; b = 4.1; c = 35.3; spicule length = 56 μ; buccal cavity = 34×19 μ; supplements = 14.

Labia of female prominently flared, papillae distinct. Buccal cavity large, about half as wide as long, with thick, prominently striated walls. Posterior

refractive ring conspicuous, appearing laminated. Dorsal tooth massive opposed by two large submedian teeth. Ovaries two, reflexed one-half distance to continuous vulva. Conspicuous valve between uterus and oviduct. Several small papillae forward and behind muscular vagina. Sperm found in reproductive tract of one female. Tail long, arcuate; suddenly decreasing in size a short distance behind slightly raised anus. Caudal glands three, in tandem, leading into common duct opening into armed terminal spinneret.

Male.—Details of male head area and tail same as those of female. Testes double, slightly reflexed, containing many sperm. Spicules long, curved, and of medium thickness. Gubernaculum trough-shaped, bifid, and about one-third length of spicule. Accessory pieces with bifurcated distal ends. Supplements equidistant and slightly raised above level of cuticle.

Differential diagnosis.—This species resembles *M. sigmaturus* but differs markedly in length and structure of the tail, in details of buccal cavity, and especially in the prominently flared labia.

Habitat.—Soil in pear orchard.

Geographical distribution.—Placerville, California, U.S.A.

Holotype female.—Collection No. 286, collected by M. W. Allen from pear orchard soil, Placerville, California, deposited with the University of California Collection, Davis, California.

Allotype male.—Collected by S. A. Sher from soil, Placerville, California, in 1951, deposited with the University of California Collection, Davis, California.

Paratypes (male and females).—Collection Nos. 216 (4b), 286, and 277, collected by M. W. Allen from pear orchard soil, Placerville, California, deposited with the University of California Collection, Davis, California.

Mylonchulus striatus (THORNE, 1924) ANDRÁSSY, 1958
(Figs. 69–75)

Canadian Specimen

(*1 female*).—L = 1.10 mm; a = 21.8; b = 3.7; c = 50.0; V = 64%; buccal cavity = 20×12 μ; tail length = 0.022 mm.

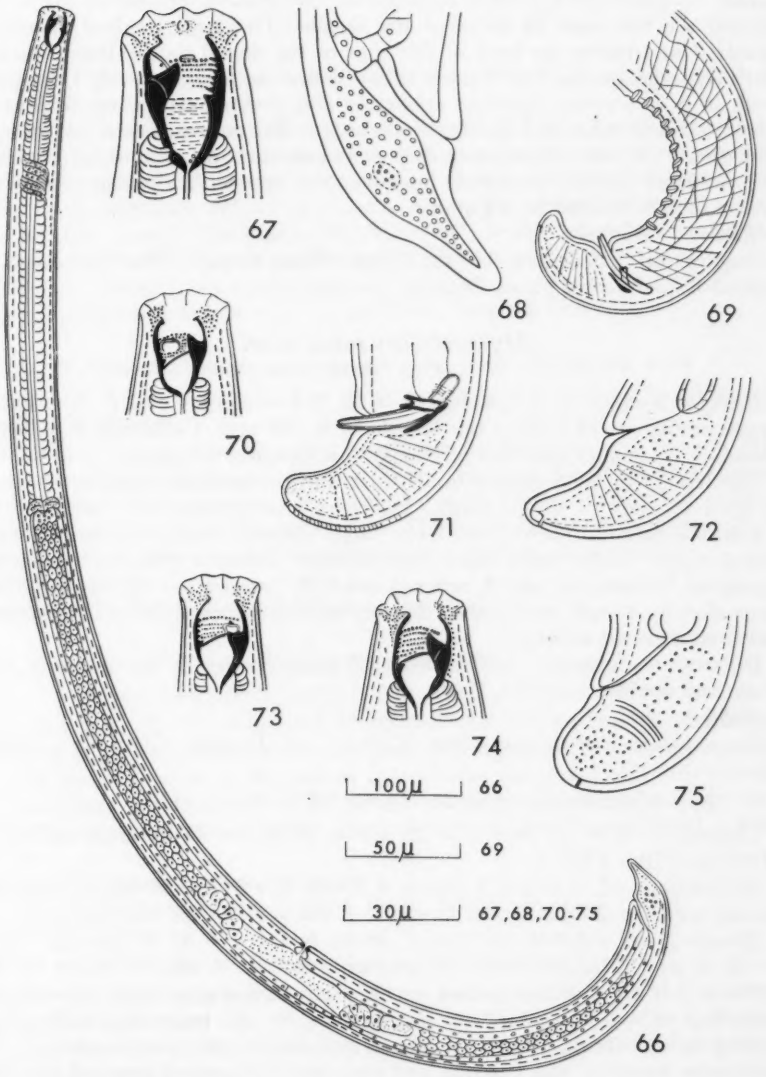
Netherlands Specimens

(*3 females*).—L = 1.12 mm (1.10–1.15); a = 29.4 (28.2–30.2); b = 3.6 (3.4–3.8); c = 42.2 (30.6–55.0); V = 64% (63–66); buccal cavity = 18–19 × 11–12 μ; tail length = 0.028 (0.020–0.036).

(*1 male*).—L = 1.10 mm; a = 34.4; b = 3.5; c = 34.4; spicule length = 37 μ; buccal cavity = 18×13 μ; tail length = 0.032 mm; supplements = 12.

The Canadian specimen fits Thorne's description (46) fairly well except for the absence of striations in the cuticle. The amphid aperture width in the Canadian specimen (about 3.2 μ) is much less than that of Thorne's specimens (about 5.0 μ). The buccal cavity of Thorne's specimen measured approximately 30×17 μ. An egg in the Canadian female measured 92×37 μ.

The Netherlands specimens also lack distinct striations in the cuticle. However, in other anatomical details they agree with Thorne's description of *M. striatus*. An egg in one of the females measured 90×30 μ.



FIGS. 66–68. *Mylonchulus solus* n. sp. 66. Female. 67. Female head. 68. Female tail.
FIGS. 69–73. *Mylonchulus striatus* (specimens from the Netherlands). 69. Male tail showing arrangement of supplements. 70. Male head. 71. Male tail showing spicule and bifurcated accessory piece. 72. Female tail. 73. Female head.
FIGS. 74, 75. *Mylonchulus striatus* (specimen from Canada). 74. Female head. 75. Female tail.

Male.—This is the first male recorded for *M. striatus*. Details of the head and tail are the same as those of the female. The large amphid aperture is located just below the level of the apex of the dorsal tooth. Dorsal tooth relatively large, opposed by two small but distinct submedian teeth. Denticles small, in 5 or 6 rows. Posterior refractive ring distinct. Intestine distinctly tesselated, cells large and distinctly nucleated. Testes two, containing many small sperm. Spicules short, accessory pieces obscure, distal ends bifurcated. Tail arcuate, bluntly rounded; rudimentary spinneret opening dorsally. Cuticle faintly striated in tail area.

Habitat.—Grass sod.

Geographical distribution.—Utah, U.S.A.; West Kapelle, The Netherlands; Central Experimental Farm, Ottawa, Canada.

Mylonchulus solus N. SP.

(Figs. 66–68)

Holotype female.—$L = 1.40$ mm; $a = 25.4$; $b = 3.8$; $c = 35.0$; $V = 68\%$; buccal cavity = 34×18 μ; tail length = 0.040 mm. Collection No. 1178, Canadian National Collection of Nematodes, Ottawa, Canada.

One female in good condition. Head truncate, amphids narrow, situated at level of apex of dorsal tooth. Denticles small, numerous, arranged in 6 rows. Submedian teeth relatively large. Dorsal tooth relatively large, buccal cavity large, walls thick, prominently striated. Intestine distinctly tesselated. Ovaries two, short, reflexed one-half distance to continuous vulva. Anus slightly raised, tail conoid then cylindroid in posterior half. Spinneret and caudal glands absent.

Differential diagnosis.—Differs from all other species in this genus in tail shape and details.

Habitat.—Sod.

Geographical distribution.—Ste. Gedeon de Beauce, Quebec, Canada.

Mylonchulus subsimilis (COBB, 1917) MEYL, 1957

(*Juvenile*).—$L = 0.8$ mm; $a = 26.3$; $b = 10.0$; $c = 25.0$; buccal cavity = 17×12 μ. (After Cobb.)

(*20 females*).—$L = 0.9$–1.3 mm; $a = 32$–35; $b = 4$; $c = 30$–40; $V = 63$–66; buccal cavity = 20×11 μ; tail length = 0.045 mm. (After Meyl.)

(*2 males*).—$L = 0.9$–1.3 mm; $a = 36$–40; $b = 4$; $c = 37$–42; spicule length = 45–50 μ; tail length = 0.050 mm; supplements = 10–11. (After Meyl.)

Cobb (14) erected this species on the basis of a single young female. He remarked that it resembled *M. brachyuris* and *M. minor* but differed in having no spinneret. Meyl (32) redescribed the female of this species from specimens found in Rio Pereque and also described and illustrated the first male found. Meyl's description and illustrations leave no doubt as to the validity of this species.

Habitat.—About roots of banana plants, stock of Iresine on dry white sand, high-placed detritus sand under *Hibiscus*.

Geographical distribution.—Paris, France; Peru?

Mylonchulus subtenuis (COBB, 1917) ALTHERR, 1958

(*Female*).—L = 1.9 mm; a = 43.5; b = 3.8; c = 33.3; V = 72%; buccal cavity = 28×18 μ.

(*Male*).—L = 1.8 mm; a = 45.5; b = 4.0; c = 33.3; spicule length = 40 μ (approx.); supplements = 14.

Cobb (14) described and illustrated (head and tail of female) this species in detail. Anus raised and conspicuous with anterior lip somewhat massive and overhanging. Tail arcuate, conoid. Tail of male like that of female but diminishing suddenly behind the anus. Accessory pieces bifurcated. Anal muscles prominently developed. Resembles *M. minor* but differs in having thicker walls of the pharynx and larger amphids.

Habitat.—About roots of plants.

Geographical distribution.—Arlington Farm, Virginia, U.S.A.

Mylonchulus subterraneus (SCHNEIDER, 1940) ANDRÁSSY, 1958

(*1 female*).—L = 1.08 mm; a = 29.0; b = 3.8; c = 35.0; V = 60%; tail length = 0.031 mm.

Schneider (38) erected this species on the basis of a single female. His illustrations show the female tail and ovary. He remarked that this species differed from *M. brachyuris* in the structure of the mouth cavity but neither illustrated nor described these differences. The gonad is unpaired and prevulva, which separates this species from closely related species in this genus.

Habitat.—Soil.

Geographical distribution.—Ljubljana, Jugoslavia.

Species Inquirendae

Mylonchulus denticulatus (COBB, 1917) ANDRÁSSY, 1958

Micoletzky (33) described and illustrated this species from a mutilated specimen of which the posterior end, including the gonads, was missing. He omitted giving it a name because of these circumstances. Cobb (14) named this specimen *M. denticulatus*. These are the only records on this species. I feel that the material available was inadequate for the erection of a new species.

Habitat.—Among fresh-water algae.

Geographical distribution—Zambezi River, South Africa.

Mylonchulus micrurus (COBB, 1917) ANDRÁSSY, 1958

Cobb (14) appears to have described this species on the basis of first-stage juveniles. He remarked that *M. micrurus* resembled *M. brevicaudatus*. Thorne (46) examined about 30 juveniles of this species from Wisconsin. I feel that this is not a valid species.

Andrássy (4) considers *M. brevicaudatus* a synonym of *M. micrurus* on the basis of similarity of buccal cavity and tail structures. However, comparison of adults with juvenile specimens through illustrations and literature does not seem sufficient to justify this synonymy.

Habitat.—About litchi roots, and in sphagnum moss.

Geographical distribution.—Fukien, China; Community Point, Wisconsin, U.S.A.

Mylonchulus polonicus (STEFANSKI, 1915) ANDRÁSSY, 1958

Stefanski (44) erected *M. polonicus* on the basis of two juveniles. His illustration of the tail region is adequate but that of the head is very inadequate for comparison with other species. *M. montanus* resembles *M. polonicus* in details of tail structure but, because of inadequate illustration of the buccal cavity, comparison between the two is impossible. I disagree with Andrássy's (4) synonymy of *M. montanus* with *M. polonicus.*

Habitat.—In vegetable detritus.

Geographical distribution.—Czarna River, Poland.

Mylonchulus sparsus (COBB, 1917) ANDRÁSSY, 1958

Cobb (14) based his description and illustrations of this species on a molting juvenile. Since that time no one has recorded finding this species. I feel that Cobb had insufficient material to justify the erection of a new species.

Habitat.—In sphagnum from greenhouse.

Geographical distribution.—In greenhouse, United States Department of Agriculture, Washington, D. C.

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THE PRACTICE AND THEORY OF BIOLOGICAL CONTROL OF INSECTS IN CANADA[1]

A. L. Turnbull and D. A. Chant[2]

Abstract

This paper constitutes a "stocktaking" of biological control in Canada. The success or failure of each of 31 Canadian biological control projects is assessed. Past experiences in Canada are analyzed, and recommendations for future work are made. The meaning of the term "control" is defined in an economic context. Lloyd's (1960) suggestion regarding the influence of host plant longevity on the success of biological control is rejected, and a new concept based on the type of injury inflicted by the pest is substituted. There is a discussion on the philosophy of biological control and some of the popular misconceptions regarding it. Some dangers of biological control are suggested. Smith's (1929) analysis of the population effects of multiparasitism is challenged. The simultaneous introduction of many exotic species of parasites and predators is not favored and a strong plea for more discrimination and caution in the selection of species for introduction is voiced. The need for research in this field is emphasized, and fields of research are suggested. The philosophy of a biological control research program is outlined.

Introduction

The concept of biological control is as ancient as the recorded history of man, but the practical biological control of insects dates from the early 18th century when the nature of insect parasitism was discovered. There are records of experiments in biological control from the early 1800's, and the first issues of Zoological Record (circa 1865) show that contemporary zoologists were aware that some of their crop protection problems resulted from the failure of parasites and predators to control insects in certain seasons. The first striking success in biological control was that of the cottony cushion scale in California orange groves in 1889 by *Rodolia* (= *Vedalia*) *cardinalis* (Muls.), a coccinellid. This success was so spectacular that for several years many agriculturists, and not a few entomologists, were convinced that in biological control they had found a panacea for all insect problems. Other forms of control were neglected and much money and effort were wasted in ill-conceived efforts to find natural enemies for all insect pests. This was a dangerous period in the entomological history of North America. Large numbers of insects were collected from all over the world and released on this continent without adequate screening or quarantine. It is miraculous that these importations apparently resulted in nothing more than a waste of time and money. Repeated failures to achieve control eventually brought a return to reason.

The first suggestion for a biological control project in Canada came in 1864 from the Rev. C. J. S. Bethune, who advocated the importation of parasites

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[2]Now Officer in Charge, Research Laboratory, Research Branch, Canada Department of Agriculture, Vineland Station, Ontario.

to control the wheat midge, *Sitodiplosis mosellana* (Geh.). Bethune corresponded with British entomologists but evidently no importations were made. Since then biological control has been constantly in the minds of entomologists in Canada and many papers in the early Canadian literature advocated this method. The first shipment of foreign parasites, some eggs of the imported currant worm parasitized by a *Trichogramma* species, was received from New York State by Wm. Saunders in 1882. In 1885, James Fletcher, the first Dominion Entomologist, received several shipments of parasites from Europe via the United States through the courtesy of C. V. Riley. But it was not until 1910, when G. Hewitt succeeded Fletcher, that an active program of biological control was undertaken. Before coming to Canada, Hewitt studied the parasitism of the larch sawfly, *Pristiphora erichsonii* (Htg.), in England, and immediately following his arrival in this country started large-scale importations of the parasite *Mesoleius tenthredinis* (Morl.) to combat this pest. By 1919 it was apparent that a spectacular success had been achieved. Thereafter biological control became one of Canada's chief weapons against insects.

In 1911 J. D. Tothill and L. S. McLaine started a project to control the brown-tailed moth in the Maritimes, and other projects quickly followed. In 1923 the European corn borer began to devastate crops in Ontario and a program of biological control was organized under A. B. Baird. Because of the rapid spread of the borer this soon became the major biological control program in Canada.

From 1929 to 1955 biological control in Canada was dominated by A. B. Baird. Under his guidance it became a separate branch of economic entomology that required special facilities, techniques, and skills, and officers whose training and interests were largely focused on this field (8).

Baird's outlook was essentially practical and he never wavered from his conviction that his ultimate responsibility was to Canadian foresters and farmers. His major targets were insects accidentally introduced into Canada without their native natural enemies. Such species frequently multiplied rapidly in their new environment and became very destructive. It was assumed that this rapid multiplication resulted from the absence of natural enemies and that the obvious cure was to introduce as many and as varied a selection of these as possible.

Biological control attempts were not confined to foreign pests. Parasites of native pests were transferred from one part of the range of the pest to another from which they were assumed to be excluded by natural barriers. Also, propagation was used to increase the numbers of some native parasites.

Such projects were carried out with great tenacity. Baird and his staff imported, reared, and released almost a billion parasites against 60 insect pests and a weed. Twenty-nine of these were minor pests of local or temporary significance against which surplus entomophagous organisms, primarily obtained for use against one of the major pests, were released. As there were rarely any follow-up studies on these releases it is now virtually impossible to assess the results. We have concluded that they cannot be called valid biological control projects and have not considered them in this paper. The remaining 31 projects were determined efforts to establish biological control. In the following pages we attempt to pass judgment on their success or failure.

Meaning of Control

As the objective of these projects was to "control" insects, we should first consider the term "control" and indicate its meaning to us. In economic entomology, "control" usually applies to pest species. Insects may be called pests for many reasons, but the principal is that they damage commodities that man requires or wants. A certain amount of such damage can be tolerated, but occasionally a species becomes so abundant, or attacks a commodity so valuable, that the damage becomes intolerable. We then attempt to restrict the damage and say that we try to "control" the insect. What we mean is that we try to "control" the amount of damage done by the insect.

There are many ways by which insect damage may be restricted, some of which are concerned with the limitation of the insect population causing the damage, but many of which have little or nothing to do with the insect population. The commonest way, and perhaps currently the most effective way, of restricting damage is the application of toxic chemicals, but this method rarely produces more than a transient reduction of the pest population. Was the mean population of Oriental fruit moth lower in the 1950's after a decade of intensive application of organic insecticides than in the 1930's before these substances became available? The damage to peaches was less; thus damage was controlled, but the insect population was not. It is even more misleading to talk of controlling the western wheat stem sawfly by developing sawfly-resistant wheat, or of controlling the European corn borer by developing strong-stemmed hybrid corn. These, and other methods of control, such as insect repellents, insect barriers, and some cultural practices, are not primarily aimed at reducing pest populations; they are intended to restrict damage even though the pest remains abundant. Any changes in the pest population resulting from these practices are incidental to the control of damage.

It is probably too late to reform this usage of the term "control" in economic entomology. As currently used the term is broadly understood: an insect is controlled when it does not cause economically intolerable damage, and it is uncontrolled when it does. This is the definition we accept. But one should bear in mind that this meaning of control has little to do with populations or with the abundance of insect species and is without biological significance. A single dermestid larva may cause economic damage if it eats a hole in a mink coat; in that particular circumstance it is uncontrolled. On the other hand 10,000 Collembola per square meter of soil may cause insignificant damage and therefore are controlled.

There is another meaning of the term "control" that is applied in population ecology. In this context, "control" refers to the processes that determine the abundance of an organism at any given time or place. It is axiomatic that there are finite limits to the abundance of all organisms at all times. Thus, in this usage of the term, all organisms must be controlled at all times. Controls are not lacking because a species achieves an abundance higher than suits the convenience of man.

In the practice of biological control both meanings of control are appropriate. We are primarily concerned with economic control of insect damage, but we are also concerned with those agents that limit the numbers of individuals in all populations. These two concepts are quite different and the dual use of the

single term "control" to apply to both has inevitably led to confusion. Milne (137) stated the case very clearly but we feel that he applied the term "control" to the wrong concept. We would reserve control for the economic concept, i.e., the control of damage. For the influence of agents that limit the numbers of individuals in a population we prefer the term "regulation" as applied by Lack (109). Without subscribing to any particular theory of how these agents work, we feel that this term is more descriptive of the influence they exert. We will, therefore, use regulation to mean this latter concept.

Franz (69) seems broadly in agreement with us on the use of control in the economic sense. We agree with Franz that the definition of Stern *et al.* (195) confuses the two concepts by pooling them. Franz suggests that the meaning of control be restricted to situations in which the numbers of an organism were reduced by the efforts of man. We cannot agree to this definition on two counts: we maintain that control is complete when economic damage is not significant, regardless of any reduction that may occur in the abundance of the potential pest species; and we do not believe that an organism is uncontrolled because man played no part in establishing control. In our definition, control is a state of affairs that can be brought about by man or can occur naturally. Such a state is usually brought about or maintained by agents called controls or, more correctly, controlling agents. Many of these same agents, or others like them, are at work regulating all populations at all times. If they regulate the population of a potentially harmful organism at a level at which it does no economic damage, such agents, in this special circumstance, are agents of control. But though regulating agents may by chance sometimes coincide with control agents, the two are not synonymous. There are many controlling agents that by their nature could never be regulating agents, and vice versa.

Franz objects to a term in which "the result of an action is used for definition". This applies to our usage of the term control. If we take certain measures with the objective of achieving control, but fail to do so, Franz claims we cannot say we have engaged in control work. Therefore we must wait until we have completed our work and demonstrated that we have achieved control before we can define what we are doing. This we feel is a technicality. If we do certain things for the purpose of controlling insect damage, we can say we are engaged in control work whether or not our efforts are successful.

To define control unfortunately does not eliminate the difficulties involved in assessing the success of a control project. Economic conditions vary enormously from place to place and from time to time, as well as with the purpose for which the damaged commodity is intended. Thus the success of a project cannot be expressed in absolute terms if tolerability to damage is to be the criterion. But any demonstrable reduction of damage is of some value whether or not the reduced damage is economically tolerable at all times or places. Thus, for our purposes, we will consider any measurable reduction of damage as a degree of control, but for complete control we will require that the damage be reduced to a level that is unlikely to be intolerable in any foreseeable economic situation.

Evaluations of Biological Control Attempts in Canada

The reviews given here are very brief; comprehensive reviews by McLeod,

and McGugan and Coppel, will shortly be published by the Commonwealth Institute for Biological Control in the form of Technical Communications, and these works need no duplication. Except for a few cases where records of this Institute provided the only available information, our reviews and conclusions are based on published statements of workers engaged on the projects. It must be recognized that assessment of most biological control projects must be intuitive and cannot be supported by scientifically acceptable evidence. Such assessments will invariably be subject to wide differences of opinion. The following assessments are opinions and thus we do not expect universal agreement on them.

Grasshoppers

The many species of grasshoppers that are native to Canada are attacked by a wide variety of natural enemies, including mammals, birds, predacious and parasitic insects, and pathogens (172). Nevertheless, an arrangement was made with Argentina to exchange parasites. The first shipment was received in Belleville in 1938 and others arrived in 1939. They were mostly of *Sarcophaga caridei* Brethes* but a few individuals of related species were included. The parasites were propagated and were released at two points in Ontario but seemed to have little or no effect on grasshopper densities.

Mantis religiosa L. was first recorded in North America in 1899 and in Ontario in 1914 (105). It sometimes feeds extensively on native grasshoppers, and in places it is very abundant. Specimens from Ontario were released in western Canada, where the species did not occur, but in neither the east nor the west can it be claimed that this predator is effective in controlling grasshoppers.

This project, then, was a failure.

European Earwig, Forficula auricularia L.

Although the European earwig is now cosmopolitan in most subtropical and temperate regions of the world, it probably is not endemic to North America, but arrived early in the century via ocean trade. By 1928 there were large populations of earwigs on Vancouver Island, the lower mainland of British Columbia, and the coast of Washington and Oregon. From 1928 to 1931, colonies of the dipterous parasite *Bigonicheta spinipennis* (Meigen) were obtained from England and liberated in Vancouver. By 1934 no evidence of parasite establishment was obtained and in 1934 new stocks of *B. spinipennis* were obtained from France through the United States Department of Agriculture at Portland, Oregon. Establishment was almost immediate. Introductions from this source continued annually until 1938, and colonies were established throughout the infested areas of British Columbia. Additional releases after 1939 were from field-recovered stock. In 1943, 70.7% parasitism of a small collection of earwigs was reported from the city of Vancouver (177). McLeod (132) collected earwigs at 17 widely distributed points throughout the province; *B. spinipennis* was present in all agricultural areas of Vancouver Island, the lower mainland, and the Okanagan Valley with the exception of the Kelowna area where 46 earwigs collected in September yielded no parasites.

*The taxonomic position of all insects discussed in this paper may be found in the Appendix.

At this season all parasites would probably have emerged. Parasitism on Vancouver Island ranged from about 11% to 50%, with a mean of 15.6%, and on the mainland from 0% to 30% with a mean of 12.6%. As the parasite has two generations per year while the host has only one, the aggregate annual parasitism was undoubtedly much higher than indicated by these figures.

An assessment of the success of this project presents unusual difficulties. The parasite is firmly established and exacts an annual toll of earwigs, but there are no precise data to indicate what effects, if any, this mortality exerts on the host population. But there seems little doubt that present earwig populations are much lower than those of the 1930's. The number of complaints received regarding earwig infestations has greatly decreased, and the public no longer seems to consider earwigs a serious nuisance.

The economic status of *F. auricularia* is not clear. Earwigs have long been suspected of causing direct damage to flowers, fruits, foliage, and roots of many cultivated plants. Undoubtedly, at high population levels they occasionally cause appreciable damage to orchard and garden crops (3, 49, 75, 82, 125). Much of the plant food, however, is of no economic consequence, consisting of mosses, lichens, and fungi (49). A large part of the remainder probably comes from vegetative parts of plants and, except in unusual circumstances, only a small part from marketable produce. On the other hand, all authors agree that earwigs feed on animals as well as plants. In one of the few quantitative studies, McLeod and Chant (133) found that about equal quantities of plant and animal food were consumed. Each earwig on the average ate 8–9 insects per night, including a wide variety of injurious aphids and scales. One of us (D.A.C.) examined the guts of a large number of earwigs from derelict apple orchards in southern England. The recognizable materials found therein consisted of green cells from leaf epidermis, nymphal stages of mites, and skeletons of various orchard insects. The chief vices of earwigs appear to be their formidable appearance, their habit of congregating in and about dwellings, and their common occurrence in garbage and other accumulations of refuse. The initial abhorrence thus engendered easily leads to a verdict of guilty on the charge of economic damage. But earwigs have at least potentialities of being definitely beneficial by attacking many harmful insects.

In view of the nuisance value of earwigs in and around dwellings, attempts to prevent excessive numbers are probably desirable but at the same time healthy and vigorous populations should be preserved. *B. spinipennis* seems to have accomplished this happy compromise very successfully in British Columbia. This project, therefore, is considered a success even though the same degree of regulation of a pest insect that has no predatory potentialities might not be considered satisfactory control.

European Fruit Lecanium, Eulecanium coryli (L.)

Eulecanium coryli (L.) probably entered British Columbia on nursery stock from Europe about 1903 (88). By 1925 it was a serious pest of ornamental and native forest trees near Vancouver. Most deciduous tree species are vulnerable. From 1925 to 1931 annual applications of oil sprays were necessary to protect boulevard and park trees in Vancouver. Though the sprays provided temporary relief, reinfestation from surrounding forests was almost immediate

(81). By 1930 the infested area covered about 200 square miles.

In 1927, two shipments of the parasite *Blastothrix sericea* Dalm. were received from England. In mid-July, 1928, 263 adults were released in North Vancouver. In 1929 an additional four shipments were received and 779 adults were released at the same place.

The parasite was first recovered in 1930 and was found to have dispersed over about 20 square miles. Parasitism varied from 85% at the points of liberation to about 1.0% at the margins of the dispersal area. In 1930, the parasite was redistributed from field-recovered material to nine points covering the entire city of Vancouver. By the autumn of 1931 it was fairly evenly distributed over about 150 square miles. Parasitism ranged between 40% and 95% and the spray program in the city was stopped. By 1932 the lecanium scale was thoroughly controlled. Annual checks showed the following trend: 1930, an average of 35 scales per foot of twig; 1931, an average of 24 scales per foot; 1932, a maximum of 2 scales per foot with most trees virtually free of scales (81).

Scale populations remained low for several years. Brief references in the Canadian Insect Pest Review to minor scale damage and the prevalence of *B. sericea* from 1937 to 1940 give no indication of any serious changes, although the scale continued to spread to the east as far as Chilliwack and west to Vancouver Island (88). McLeod (135) reported continuing control of the scale to 1953, when about 40% of the overwintering scales were parasitized. As *B. sericea* has two generations to each one of its host, total mortality caused by *B. sericea* must greatly exceed this figure. As there have been no reports of damage to trees since 1953 we assume that control continues.

Graham and Prebble (88) made a comprehensive study of the relationships between *B. sericea* and its host. They found that factors other than parasitism caused more annual mortality to *E. coryli* than did *B. sericea*. Nevertheless, without *B. sericea*, these factors were inadequate, whereas with *B. sericea* the sum of all mortality factors was sufficient to provide control. The project was a success.

Oystershell Scale, Lepidosaphes ulmi (L.)

Lepidosaphes ulmi (L.) has been a pest of fruit and other deciduous trees in Canada for many years. The predaceous mite *Hemisarcoptes malus* (Sheiner) is native in eastern Canada, and early workers (99, 204) showed that where it occurred with the scale it was an effective control. This predator is not native to British Columbia. It was released there in 1917 at several sites in the Okanagan Valley, the Fraser Valley, and on Vancouver Island. Glendenning (79) briefly reported that the mite "has survived, and under certain conditions has effected excellent control, but has not spread greatly". Twenty-three years later, McLeod (135) reported that *H. malus* was then widely distributed in British Columbia and at times was important. He pointed out that the scale is controlled in commercial orchards by sprays primarily applied against other pests, and that the main value of the mite is to reduce the abundance of scales on native plants, thus reducing the danger of a rapid reinfestation of orchards.

No precise data are available on the value of this predator following its introduction to the west but *H. malus* has at various times been the subject of

intensive studies in North America and elsewhere. There is little doubt that this predator exerts a beneficial effect on various scale insects in many places. On circumstantial evidence we consider this project successful.

Balsam Woolly Aphid, Adelges piceae (Ratz.)

The balsam woolly aphid was first recorded in North America about 1908. For several years *A. piceae* was commonly mistaken for its close relative, *A. nusslini* (Börner) (10), and early records are therefore unreliable and confused.

About 1930 a pathological condition of balsam fir, *Abies balsamea* (L.) Miller, in the Maritime Provinces caused forest industries much concern. Trees developed a variety of symptoms including a progressive dieback that killed them in two or three years. Balch (9) published a brief account of the condition and demonstrated that *A. piceae* was the causative agent.

A. piceae has no known parasites, either in North America or in other parts of the world where it is endemic. Brown and Clark (27) reported that the species is attacked by a number of native predators, none of which has the ability to control it. The most important regulatory factor of *A. piceae* seems to be the exhaustion of local food supplies. Direct sunlight on an infested stem kills many larvae by excessive heat and heavy rains cause extensive mortality. Low winter temperatures are usually not significant mortality factors, although the onset of winter eliminates all stages but neosistens larvae. In 1934 and 1948, however, low winter temperatures are reported to have caused heavy mortality (14). Details of the life history of *A. piceae* in North America were given by Balch (10, 14) and Tunnoch and Rudinsky (207), and in Europe by Karafiat and Franz (107) and Varty (209).

In 1933 the importation of predators of *A. piceae* and of related species from Europe was started. In that year a "considerable number" of an agromyzid, *Leucopis obscura* Hal., were liberated near Fredericton and Gagetown, New Brunswick, and later were found overwintering in puparia in "very satisfactory numbers" (10). Later, predators of *A. piceae*, *A. nusslini*, *Pineus pini* (L.), and *P. strobi* (Htg.) were introduced from England. To date 12 species have been introduced. Those introductions have been well documented by Smith and Coppel (166), Smith (167), and Balch (15).

At present, neither native nor introduced predators have satisfactorily controlled the aphid although one introduced beetle, *Laricobius erichsonii* Rosenh. (Derodontidae), shows considerable promise. Near Fredericton severe infestations on trees have been eliminated by this predator, while heavy infestations remain on adjacent trees from which the beetle was removed. Three other predators have been established in New Brunswick: *Pullus impexus* (Muls.) (Coccinellidae: Coleoptera), *Leucopis obscura* Hal. (Chamaemyiidae: Diptera), and *Cremifania nigrocellulata* Cz. (Chamaemyiidae: Diptera). For various reasons none of these seems likely to produce effective control. An intensive search for new and more-effective predators is continuing on a worldwide scale.

At present this project has not achieved control of *A. piceae*. We have therefore rated it as a failure but this judgment is conditional and may have to be revised at some future date.

Woolly Apple Aphis, Eriosoma lanigerum (Hausm.)

The British Columbia Department of Agriculture reported the apple woolly aphis on Vancouver Island and the lower mainland of British Columbia in 1892. By 1912 it was "fairly common everywhere throughout the season" in the Okanagan Valley (205), and by 1916 it was a serious pest (25, 72). From 1920 to 1930 woolly aphis was the most troublesome pest of the British Columbia apple industry. It malformed trees, smutted the fruits, made picking unpleasant, and, most important, its feeding was associated with the development of the serious fungus disease, perennial canker (135). Chemical control was unsatisfactory.

In eastern North America, the aphis is heavily attacked by a native chalcid parasite, *Aphelinus mali* (Hald.). *A. mali* had been previously shipped to other continents, where it produced excellent control. In 1921 it was transferred from Ontario to the lower Fraser Valley and in 1929 to the Okanagan Valley (218). Venables (210, 211) showed that the parasite achieved completely satisfactory control in a few years. "The aphid became a minor pest and remained so until DDT was generally used for codling moth control. As DDT proved innocuous to the aphid but toxic to the parasite, the aphid once again became a major pest" (135). This statement, however, has not been supported by later events. Aphid populations rose alarmingly in 1952 but collapsed the following year and the aphid has not been a serious threat in western orchards since that time. There does not seem to be any differential mortality of the aphis and its parasite attributable to DDT (136). This project was a success.

Apple Mealybug, Phenacoccus aceris (Sign.)

Phenacoccus aceris (Sign.) is native to Europe and Asia. It was first recorded in North America in Maine in 1910 and probably arrived on imported apple stock (155). It appeared in the Kootenay Valley of British Columbia in 1927 but did not become an economic pest until 1936 (123). Meanwhile it invaded the Annapolis Valley of Nova Scotia and by 1932 severe infestations were present (77). The last serious outbreak in Nova Scotia was in 1937 and 1938. Gilliatt (78) described the insect, its life history, and the damage it causes. He also recorded parasitism of up to 85% by *Allotropa* sp., later described by Muesebeck (142) as *A. utilis*.

P. aceris secretes honeydew late in the season that coats both leaves and fruit. A sooty fungus develops on this and the fruit becomes unmarketable. Chemical controls were developed both in British Columbia (121, 122) and in Nova Scotia (146).

In British Columbia no parasites were found. Beginning in 1938 *A. utilis* was collected in Nova Scotia, reared at Belleville, and shipped to Vernon, B.C., for release. Five years later the parasite was well established in the Kootenay district (124). Wishart (218) found very low mealy bug populations in both sprayed and unsprayed British Columbia orchards and reported parasitism by *A. utilis* of from 10% to 80%. Marshall (124) declared this project "one of the outstanding examples in Canada of biological control by an introduced parasite." We agree.

Citrus Mealybug, Pseudococcus citri (Risso)

Pseudococcus citri (Risso) occurs generally in greenhouses across Canada. Because growers requested parasites that attack it, two species were obtained and propagated at Belleville. *Leptomastix dactylopii* How. was received from California in 1937 and *Leptomastidea abnormis* (Grlt.) was found at London, Ontario. In 1938 and 1939 many thousands of both species were shipped to growers across Canada and satisfactory results were obtained. McLeod (130) reported that *P. citri* was controlled wherever the parasites were liberated. Though cultural practices in greenhouses make it necessary to reintroduce the parasites periodically, there is little doubt that the project was successful.

Grape Mealybug, Pseudococcus maritimus (Ehrh.)

Pseudococcus maritimus (Ehrh.) was for some years confused with *P. citri* in Canadian greenhouses. When parasites were liberated to control the latter, however, *P. maritimus* was not attacked and remained abundant. *P. maritimus* was eventually found to be widely distributed in this country. In 1939 two parasites, *Zarhopalus corvinus* (Grlt.) and *Chrysoplatycerus splendens* (How.), were obtained from California and propagated at Belleville (130). They were released in some Canadian greenhouses but did not control the mealy bug. The project was therefore discontinued. The parasites used in Canada were collected by McLeod from a host strain almost certainly different from that in Canadian greenhouses (McLeod, personal communication). The project was a failure.

Greenhouse Whitefly, Trialeurodes vaporariorum (Westw.)

Trialeurodes vaporariorum (Westw.) is a cosmopolitan pest of greenhouse plants. Workers in England reported in 1927 that the chalcid *Encarsia formosa* Gahan controlled the pest under greenhouse conditions. The parasite was distributed to many parts of the world and a stock colony arrived in Belleville in 1928. This was propagated, and in 1931 shipments were sent to selected growers to test the efficiency of the parasite. The parasite is very effective at temperatures above 70° F, but at lower temperatures its efficiency drops off rapidly. In eastern Canada during the winter months its value is slight but in the spring and summer it successfully controls whitefly. In the west the demand for the parasite usually comes in March and at that time it usually gives satisfactory control. Between 1935 and 1953, when the project was discontinued, several million *E. formosa* were supplied to growers across Canada. There is no doubt that, when used under the proper conditions, it is a successful control of the whitefly (129, 130).

The project was successful.

Brown-tail Moth, Nygmia phaeorrhoea (Donov.)

The brown-tail moth probably was introduced to North America from Europe on nursery stock. It was first recorded from Massachusetts in 1897 (108). Its hosts include most deciduous trees. Its life history and habits were described by Tothill (202), Craighead (44), and Burgess and Crossman (36).

The moth spread rapidly in the New England states and was first recorded in New Brunswick in 1902 (128). It subsequently increased rapidly in the Maritime provinces until about 1914, and then gradually declined (76).

A biological control project was initiated in Canada about 1911. Parasites from Europe that had been introduced to the United States were obtained from the United States Department of Agriculture. Three species, *Apanteles lacteicolor* Vier., *Compsilura concinnata* Meig., and *Meteorus versicolor* Wesm., were released in New Brunswick, Nova Scotia, Quebec, and Ontario. One predator, the carabid *Calosoma sycophanta* (L.), was released in the Maritimes (36, 76, 98, 99, 203). Few of the parasites were recovered from the intended host but *C. concinnata* and *M. versicolor* both became firmly established on the native satin moth, and were later used to control outbreaks of satin moth in British Columbia. *A. lacteicolor* became established on the brown-tail moth and may have contributed to the population decline that followed 1914. It is surprising that neither *A. lacteicolor* nor its host, the brown-tail moth, have been recovered in Canada for many years, though both survive in Maine, Massachusetts, and Connecticut (93).

This project must be considered a failure. Although the brown-tail moth, once considered a grave menace to deciduous trees in eastern Canada, has now apparently disappeared from this country, there is no evidence that the imported natural enemies contributed to this happy ending.

European Corn Borer, Ostrinia nubilalis (Hbn.)

The European corn borer was first recorded in North America near Boston, Massachusetts, in 1917, but was undoubtedly present on the continent much earlier. It was first recorded in Canada in August, 1920, in Welland County, Ontario, but it was probably well established in several other areas before its discovery, since by 1925 virtually all corn-growing areas of Ontario and Quebec were invaded, in spite of strict quarantine regulations. The infestations were so severe that many farmers seriously considered abandoning corn as a major crop (221). The corn borer now is present in all provinces of Canada except Newfoundland, Alberta, and British Columbia.

Biological control studies were started early. In 1920 the United States Department of Agriculture established a laboratory in France to collect and ship parasites from Europe. Some of the parasites received were made available to Canada and by 1923 large releases of European parasites had been made. A Canadian laboratory was opened to receive, propagate, and release introduced parasites. In close collaboration with the United States authorities 20 parasite species from Europe or the Orient were introduced and released (6, 7, 106). Millions of individuals were released, but almost 80% were of one species, *Bracon brevicornis* Wesm. At least three species became established, but for many years parasitism remained remarkably low and did not demonstrably contribute to the regulation of the pest population.

In the last 20 years a change has occurred in the biology of the corn borer that seems to favor parasite survival. Until about 1940 the dominant strain of corn borer in Canada had only one generation per year, though the occurrence of a multivoltine strain was noted by Crawford and Spencer as early as 1922 (45). Since 1940 the multivoltine strain has become common in western Ontario (217). With two generations per year the corn borer became much more injurious to crops. Coincident with the development of the multivoltine population, however, was an increase in parasitism. Two introduced species in

particular, *Horogenes punctorius* (Rom.) and *Sympiesis viridula* (Thoms.), seem to be increasing and may become control factors in some areas. At the present time, however, the project must be considered a failure.

Oriental Fruit Moth, Grapholitha molesta (Busck)

The Oriental fruit moth was discovered in the peach-growing areas of western Ontario in 1920. In 1925 it damaged peaches in the eastern end of the Niagara Peninsula and by 1927 had spread over the entire peninsula. By 1930 the moth was generally abundant throughout the Niagara area and up to 90% of the fruit was damaged in some districts. Further west, in Essex County, a similar infestation developed about five years later (37, 74, 191).

No adequate chemical control was devised until 1946 (60, 147, 159, 160). Thus, from the time the insect first became a serious pest in Ontario in 1925 until 1946, the only practical method of dealing with *G. molesta* was by biological control.

Early studies (4, 14, 24, 151, 169, 184, 185, 186, 187) revealed about 15 native parasites and 2 predators attacking *G. molesta* in Ontario. The two most important parasites were *Trichogramma minutum* (Riley) and *Glypta rufiscutellaris* Cress. None of the native species produced a satisfactory control. *T. minutum*, however, offered a possibility for manipulation that might have produced some protection (169).

T. minutum attacks mainly the third generation of fruit moth eggs. It was hoped that massive releases of *T. minutum* adults during the first and second generations of the host would produce a population large enough to destroy the third generation. Experimental releases were made in 1928 but were too small to produce a measurable effect (169). Much larger releases in 1929 resulted in from 12% to 60% additional parasitism (184). Further massive releases from 1930 to 1933 provided some crop protection but the parasites were unable to maintain high densities without frequent releases. In 1934, when it was apparent that other agencies were limiting the abundance of fruit moths, releases of *T. minutum* were abandoned (188).

In 1929, the hymenopterous parasite *Macrocentrus ancylivorus* Rohw. was obtained from its native New Jersey and released in the Niagara Peninsula. Recoveries were made in the year of release with parasitism as high as 57%. The parasite spread as much as three miles from release points and was well distributed over 35 square miles by the end of the year (184, 185). Releases were made annually until 1934, by which time *M. ancylivorus* seemed well established throughout the Niagara district.

M. ancylivorus proved remarkably well adapted to the peach-growing areas of Ontario (59). It is well synchronized with *G. molesta* and parasitizes a number of native insects including the strawberry leaf roller, *Ancylis comptana* (Fröh.), and the ragweed borer, *Epiblema strenuana* (Wlk.), both of which are fairly common in the area (152, 153).

M. ancylivorus attacks larvae of all generations of *G. molesta* but is most successful against the first generation. When host larvae are attacking twigs they are accessible to *M. ancylivorus* but when they enter fruits in later generations they burrow too deeply to be reached. Thus, the population of *M. ancylivorus* increases on the first host generation and the early emergents

of the second generation but declines in the third generation. This greatly reduces the number of parasites that enter winter hibernation. In early spring, however, it builds up rapidly again. It is probably augmented each spring by parasites that overwintered on hosts outside peach orchards. Moreover, *M. ancylivorus* has a very high fecundity, so that a few overwintered adults can parasitize a large number of first-generation hosts.

Prior to the introduction of *M. ancylivorus*, the most prominent parasite of *G. molesta* in Ontario was *Glypta rufiscutellaris*, which parasitized up to 60% or 70% of second-generation host larvae. This parasite, however, fails to attack first-generation *G. molesta* and thereby permits the host to become abundant early in the summer. Even heavy parasitism in late generations is not sufficient to overcome the initial advantage gained by the host. *M. ancylivorus* almost perfectly fills the gap left by *G. rufiscutellaris* but in doing so places the latter at a competitive disadvantage. The numbers of *G. rufiscutellaris* decreased progressively in each district as *M. ancylivorus* became established. From the standpoint of control this situation is not disadvantageous. *M. ancylivorus* is not interfered with by *G. rufiscutellaris*, and any mortality caused by the latter adds to that produced by the former. The two parasites are therefore complementary with *M. ancylivorus* in the dominant role (22, 23, 189, 190).

M. ancylivorus maintained reasonably satisfactory control of the fruit moth in the Niagara Peninsula until about 1945.

In Essex County the moth was still only a minor problem in 1930. Consequently only token releases of *M. ancylivorus* were made and these small colonies did not persist. By 1936 the fruit moth problem became acute and large releases of *M. ancylivorus* were made in 1936, 1937, and 1938. By the end of the third year there was completely satisfactory control with less than 1% of fruit injury reported (192). Again, however, the parasite colonies were not sustained, and by 1944 parasite numbers were very low. Further releases of *M. ancylivorus* were made in 1946 and 1947 and control was restored (23).

Thus for a period of 20 years (1928–1948) peach growers in Ontario relied exclusively on biological agents and other natural mortality factors (57, 58) to protect their crops from *G. molesta*. The results, though not perfect, on the whole were satisfactory. During the period there were fluctuations of fruit moth populations: in some years fruit damage was disappointingly high and in most years some orchards suffered heavily. But the average injury over the entire peach-growing areas was kept at an economically tolerable level.

In 1945 a severe infestation of fruit moth developed in the Niagara district. The infestation declined somewhat in 1946 with damage generally moderate, but severe in local areas. In 1947 there was another increase both in the Niagara Peninsula and in Essex County, though severe injury was again confined to a few small areas. In 1948 damage was severe in all areas and losses were greater than at any previous time. In that year spraying with DDT was started, and populations declined the following year (60). DDT received credit for this and the spray program was intensified. Standard orchard practice soon included three annual applications of DDT to control fruit moth. Strangely enough, though DDT is extremely toxic to *M. ancylivorus* (168, 213, 214), and applications of organic insecticides, including DDT, parathion, and EPN, seriously reduced *M. ancylivorus* parasitism in peach orchards of New Jersey

(1, 2, 28, 29, 30, 31, 32, 33), in Ontario parasitism by *M. ancylivorus* actually increased following the inception of chemical controls (22, 23). In spite of the secondary problems that have arisen from the use of chemicals, there is, therefore, no suggestion that such use should be discontinued in Ontario peach orchards. Putman and Herne (154), after a careful study of the orchard mite problem, concluded that the spray program must continue. As an extremely high grade of fruit is now demanded by consumers, the fruit moth must be almost exterminated annually. To achieve this the spray calendar for peaches in Ontario for 1959 recommends five annual sprays, including DDT, parathion, guthion, methoxychlor, and sevin.

These heavy applications of chemicals do not in themselves provide the desired degree of control (154). Dustan (1959, *in litt.*) made these comments: "theoretically, a complete, extensive spray program would give almost perfect control in the absence of parasitism, but in practice our program never gives nearly perfect control, and in the absence of appreciable parasitism often would not give commercial control."

The survival and continued effectiveness of *M. ancylivorus* in heavily sprayed peach orchards is of great interest. Pielou and Glasser (148) showed that a laboratory population of *M. ancylivorus* developed a degree of resistance when exposed to DDT. Boyce and Dustan (23), however, declare that there is no evidence that *M. ancylivorus* has developed any significant resistance in Ontario orchards. A possible source of the continuing population of *M. ancylivorus* in sprayed peach orchards may be *G. molesta* and other hosts in nearby unsprayed gardens, orchards, and fields (Dustan, 1959, *in litt.*).

Populations of *M. ancylivorus* have varied widely since the introduction of chemical controls, and the variations seem to bear no relation to the intensity of chemical application. For instance in Essex County in 1956 the average per cent parasitism of first and second generations of twig-infesting larvae was 35.8 and 31.4 respectively; in 1957 comparable figures were 20.5 and 24.3%; in 1958, without any appreciable change in the spray program in either Essex County or the Niagara district, parasitism in Essex County dropped to 0.5% for the first generation and 4.1% for the second; in the Niagara district comparable figures for the same year were 46 and 76%. In 1959, in spite of the introduction of chemical measures against first-generation larvae to supplement those already in use against larvae of the second and third generations, parasitism increased in Essex County to 2.0% of the first generation and 12.0% of the second. In Niagara, where a similar intensification of the spray program was instituted, parasitism was practically unchanged from the previous year. It therefore appears that "variations in parasitism . . . result more from the operation of natural ecological factors than from chemical control measures" (Boyce, 1959, *in litt.*).

G. rufiscutellaris has virtually disappeared from peach orchards following the use of chemicals.

There are many features of this project that are not understood. In our opinion these features are of great significance in the field of integrated control programs, and warrant much more thorough investigation.

Though from 1929 to 1948 biological agents were reasonably successful in controlling the peach moth in Essex County and the Niagara Peninsula, since

1948 they have not been able to maintain a satisfactory control by themselves. Chemical insecticides now constitute the main control of this pest, supplemented at times by biological agents. This project, therefore, though it may once have been called successful, can now at the best be called only a partial success.

Codling Moth, Carpocapsa pomonella (L.)

The codling moth, *Carpocapsa pomonella* (L.), has been present in North America for well over 100 years, its establishment in local areas closely following that of the fruit industry. In British Columbia, for example, it was first recorded in 1900 on Vancouver Island and gradually spread to the interior (135). The earliest Canadian attempt at biological control of this pest involved the parasite *Ascogaster quadridentatus* (Wesm.) (=*carpocapsae* Vier.), which was probably introduced to Eastern Canada with its host. Boyce (18) reported that this parasite was collected in Ontario and shipped to British Columbia in 1935, and subsequently was recovered on banded, unsprayed apple trees in the latter province. It was established in the western United States some years earlier (18) and the prospects of establishing it in western Canada seemed good. However, McLeod (135) found that, though establishment was successful, the parasite did not become abundant and the attempt was therefore a failure.

A more detailed study of parasites of the codling moth in Ontario followed this failure (19) and a number of predators and parasites were recorded. None seemed useful, however, and a search was initiated in Europe. Simmonds (164) reported that parasitism in France, though high, did not prevent nearly all suitable fruit from being attacked. Despite this somber finding Simmonds recommended introducing to Canada nearly all the European parasites, a project that was prevented by the outbreak of war. Only two species, *Apistephialtes caudata* (Ratz.) and *Cryptus sexmaculatus* Grav., were collected and shipped to Canada prior to the German invasion of France and two other species, *Elodia tragica* (Meig.) and *Pristomerus vulnerator* (Panz.), were shipped from England in 1943 and 1944. These were liberated, the ones from France in both the east and the west and those from England only in the east. Boyce (21) could not estimate the importance of these parasites as he felt it was too soon to expect recovery. It now appears that none of the species became important in Ontario. McLeod (135) reported that *A. caudata* and *C. sexmaculatus* did not become established in British Columbia, and we must conclude that up to the present all efforts to achieve biological control of the codling moth in Canada have failed. Further work is planned.

A number of explanations for the apparent failure of *A. quadridentatus* have been suggested. Among these are: a hyperparasite, *Perilampus* sp., greatly reduces its effectiveness (21); sprays are detrimental to it (20, 41, 56); and the host larvae vacate infested apples at night to spin their cocoons, thus exposing themselves to attack at a time when *A. quadridentus*, a diurnal, positively phototactic insect, is inactive (71).

From 1952 to 1956 attempts were made to control the codling moth with a bacterium (193, 194). These were not successful.

This project was a failure.

Satin Moth, Stilpnotia salicis (L.)

The satin moth, *Stilpnotia salicis* (L.), has a nearly circumpolar distribution in the northern hemisphere but was not recorded from British Columbia, the only place in Canada where it is an economic pest, until 1920. It was found in New Westminster and rapidly spread to poplars in the entire Lower Fraser Valley where it caused severe damage in 1922. Glendenning (80) recorded that in 1923 the population was somewhat reduced for unknown reasons. He surveyed the natural mortality of the moth in this area and found that winter mortality of hibernating larvae is severe and that a number of native parasites and some predators, especially birds and bats, attack it. Winter mortality was caused mostly by a fungal disease which appeared in the second or third season of infestation and subsequently reduced defoliation from 100% to 50% or less. Two species of parasites in particular were noted, both tachinids. Both were fairly common in 1922 (up to 65%), scarce in 1923, and apparently absent in later years.

Four species of parasites, *Compsilura concinnata* (Mg.), *Apanteles solitarius* (Ratz.), *Epteromalus nidulans* (Thoms.), and *Meteorus versicolor* Wesm., were introduced from New Brunswick and Massachusetts from 1929 to 1934 (135). All species parasitized the satin moth in the eastern United States.

Of the four, only *E. nidulans* failed to establish. *A. solitarius* spread rapidly. McLeod (135) recorded that the satin moth in the lower mainland of British Columbia declined in numbers following these introductions and that no further outbreaks occurred. In 1951 McLeod collected the moth at Vancouver and found a 64% parasitism by *C. concinnata*, *M. versicolor*, and *A. solitarius*. He concluded that the introduced species continued to be effective, and we agree that this is the case in the lower mainland region of the province.

Condrashoff (42) stated that the satin moth had spread to the interior of British Columbia, and Arrand (5) noted that it is now a pest of some importance in the interior, attacking aspen, white poplar, Lombardy poplar, Carolina poplar, and willows. There have been no reports regarding the presence or absence of the introduced parasites in these regions. It seems obvious that if these parasites have not followed their host an effort should be made to establish them in the interior.

We rate this project as a success.

Spruce Budworm, Choristoneura fumiferana (Clem.)

Choristoneura fumiferana (Clem.) is native to North America and is found throughout Canada wherever balsam fir, white spruce, alpine fir, or Douglas fir occur. Several severe outbreaks occurred in the 19th century and early in the 20th. There was a serious outbreak starting in the late 1930's in Ontario and a more recent one in New Brunswick and Quebec. As a large volume of literature is available on these infestations and on attempts to control them, we will not review them here. Biological control was first considered in the early forties but importation from other countries was prevented by World War II. Initially, therefore, parasites were brought from British Columbia to eastern Canada. In British Columbia budworm populations rarely achieved outbreak proportions and it was felt that populations in that province might be under control from natural enemies. About 20 species were collected in British Co-

lumbia and released in Ontario, Quebec, Newfoundland, and New Brunswick. As most of these species already occurred in the eastern provinces, this phase of the project was largely one of carrying coals to Newcastle.

After the war, parasites of a variety of lepidopterous hosts were imported from Europe to Canada. In all, 13 species of parasites were released. The hosts from which they were collected were as closely related to the spruce budworm as *Choristoneura murinana* (Hbn.) and as remote as *R. buoliana* (Schiff.).

It is doubtful whether any parasitic species not previously present became established in Canada as a result of this work (127). The spruce budworm remains a pest of primary importance in eastern fir and spruce forests. The project was a failure.

European Pine Shoot Moth, Rhyacionia buoliana (Schiff.)

Rhyacionia buoliana (Schiff.) was first recorded in Canada in 1925. It feeds on all native pines and on several introduced European pines used as ornamentals and for plantations. Damage is most severe in plantations in southern Ontario. An attempt to control the shoot moth by introducing parasites was undertaken in the late 1920's. The first parasites were received in 1928 and releases of at least 10 species have been made since then at irregular intervals. These parasites originated from other parts of Canada, from England, and from Europe (43). Some at least of the introduced species became established. In addition, the shoot moth is now attacked by a complex of indigenous species (212).

It is more difficult than usual to assess this attempt at biological control. Young plantations are usually infested most heavily. As the plantations mature the levels of infestation decline and few attacks occur in mature stands. It is, therefore, nearly impossible to follow an infestation after parasite releases to determine what effect, if any, they may have had. It is difficult to determine how much of the subsequent shoot moth decline is due to the parasites and how much to other factors. We were tempted to rank this project as a partial success because several introduced parasites have become established and probably aid in regulating shoot moth abundance. However, these parasites were introduced to Canada to prevent damage to young pine plantations. As they have not succeeded in this we must regard the project as a failure. Work is still in progress, however, and the picture may improve.

Pea Moth, Laspeyresia nigricana (Steph.)

The pea moth entered Canada from Europe prior to 1893 (64). It became a pest of pea crops in the lower Fraser Valley in British Columbia, in southern Ontario, and in the Gaspé region of Quebec, southern New Brunswick, and the Annapolis Valley of Nova Scotia. It became a major pest in British Columbia during World War II when ripe and seed peas were grown to replace those normally supplied from Europe. Occasionally from 80% to 100% of the pea pods were infested. In Nova Scotia pod infestation was as high as 70%. Throughout Canada, the insect was almost free from attack by native or naturalized parasites (215) and by 1938 no practical chemical control had been found (38).

A biological control program was started in 1936 using the parasites *Ascogaster quadridentatus* (Wesm.) (= *carpocapsae* Vier.) and *Macrocentrus*

ancylivorus Rohw. which, though present in Ontario and the Maritimes, were not known to occur in British Columbia and Quebec where the pea moth was present. Both species were liberated in 1936 and 1937 but apparently failed to become established.

Cameron (38) found that three species of parasites, *Ascogaster quadridentatus* (Wesm.), *Glypta haesitator* Grav., and *Angitia* sp., destroyed up to 60% of overwintering host larvae in England, where *L. nigricana* is a minor pest. He recommended their introduction to Canada. The first shipment arrived in Canada in 1936 (215, 216) and *A. quadridentatus* and *G. haesitator* subsequently became established in British Columbia, Quebec, and Nova Scotia. Wishart (218) showed that parasitism in British Columbia increased steadily from 1941 to 1945, and reached a peak of over 80%. Crop damage dropped from over 80% in 1945 to 35% in 1946.

Shortly after this, two events occurred that obscured the situation. Firstly, with a return to peacetime conditions mature pea crops were no longer economically attractive in British Columbia and their production ceased. The main pea crops now grown in the area are harvested while green. This restricts the population of the pea moth because the insect cannot mature on plants harvested before ripening (135). Secondly, a flood in 1948 inundated most of the commercial pea-growing lands in the Fraser Valley, with catastrophic effects on both the pea moth and its parasites. At present, however, the pea moth population survives at a low level on wild legumes such as vetch, *Vicia angustifolia* Reichard, and both species of parasites continue to attack it (Wishart, personal communication).

In view of these circumstances we cannot class this project as a complete success. The introduced parasites undoubtedly reduced the pea moth population greatly and a partial control was certainly established. But as long as crop damage remained as high as 35% the situation could not be called satisfactory. We rate the project as a partial success.

Larch Casebearer, Coleophora laricella (Hbn.)

The larch casebearer, *Coleophora laricella* (Hbn.), is native to Europe. It was introduced to North America in 1886 and subsequently caused serious injury in Canada. In 1928 it was investigated in Europe with a view to bringing its natural enemies to Canada. No insect predators were found. Of the parasites, *Horogenes nanus* (Grav.) was the most promising and Thorpe wrote "further investigations are necessary before it can be considered safe to introduce any other species" and "if this species can be established in Canada it will probably be advisable to await results before considering further introductions" (201).

Graham (84) showed that the casebearer in Canada is attacked by a number of native parasites but that these did not achieve control. The first parasites from Europe were liberated in Canada in 1931, and further releases were made from 1934 to 1939. In all, about 34,600 specimens were released. Despite Thorpe's suggestion, five species of parasites were released and all eventually were recovered in Ontario. In the early 1940's two, *Agathis pumilis* (Ratz.) and *Epilampsis laricinellae* (Ratz.), were sufficiently numerous to be

collected for recolonization. Graham pointed out that *H. nanus* did not assume the dominant role in Canada that it played in England.

Graham (86) discussed the biology of the two most abundant introduced parasites and showed how they complement one another. *A. pumilis* has one generation each year and emerges from the host larvae in June. Parasitized larvae are unable to pupate and remain in the larval stage much longer than normally. *E. laricinellae* has three generations each year. It also hibernates in the host larvae but emerges late in May. It then parasitizes overwintered host larvae prior to their pupation. At this time *A. pumilis* plays an important role: case-bearer larvae parasitized by this species are still present and are available for reparasitization by *E. laricinellae*. Control of the case-bearer apparently was not effective until the population density of overwintered *A. pumilis* reached a high level and thereby supplied *E. laricinellae* with sufficient host material in June to maintain itself during the following summer generation. *A. pumilis* dies if its host is attacked by *E. laricinellae* and parasitism of 69% by *A. pumilis* in May was reduced to 25 or 30% in late June by this competition. *A. pumilis* thus aids control by allowing the spring generation of *E. laricinellae* to survive and multiply.

In 1957 Graham (87) stated that *A. pumilis* was found in each of 56 recovery collections made in Ontario but that *E. laricinellae* had not spread more than 42 miles from any release point, an increase of only 6 miles since 1948. Parasitism by *A. pumilis* increased from south to north from 41% to 67% and then sharply decreased to 15% or less. Mortality of the case-bearer due to other factors in the winter, however, increased from south to north from 2% to 32% and thus partly compensated for decreased parasitism near the limit of its range. Parasitism by *A. pumilis* varied from 4% to 85% and was the most efficient agent of control. Apparently *A. pumilis* retains its effectiveness even at very low host density. *E. laricinellae*, however, despite the assistance provided by *A. pumilis*, is effective only where host densities were high. In general, the case-bearer population has fallen to a very low level and damage to the host tree is no longer considered serious. We rate this project as a success.

Lodgepole Needle Miner, Evagora (= *Recurvaria*) *starki* (Freeman)

What used to be known as the lodgepole needle miner, *Evagora milleri* (Busck.), is now known to be a complex of species; *E. starki* (Freeman) is the species of greatest economic importance in Canada. These species are native to North America and in Canada attack lodgepole pine in Alberta and British Columbia. The life cycle of the miner requires about 24 months, and larval development lasts about 21 of these. In this time, three needles are destroyed by each insect and, though few trees are killed, tree vigor is reduced and secondary invaders subsequently may cause death. In the late forties and early fifties an attempt was made to control the miner by importing parasites. In all, nine species of parasites were imported, two from Europe and seven from the western United States. Of these, only the European species and *Dicladocerus* sp. were not previously present in the infested areas. Further details of this work may be found in papers by Stark (178, 179, 181, 182, 183), McLeod (131), Henson *et al.* (96), Freeman (70), and Stark and Cook (180). As the miner continues to be a pest in western Canada, we have rated the attempt as a failure.

Sweetclover Weevil, Sitona cylindricollis Fahr.

The sweetclover weevil, *Sitona cylindricollis* Fahr., is a serious pest in parts of Canada. From 1951 to 1954 attempts to establish the parasite *Microctonus aethiops* (Nees) (Hymenoptera: Braconidae) from France were unsuccessful, probably because *S. cylindricollis* is not a suitable host for this parasite: in laboratory tests, over 80% of parasite larvae died shortly after hatching although they developed normally in other *Sitona* species.

Because of climatic similarities it was considered that material from Sweden might be more suited to Canadian conditions than material from France. A survey of Swedish parasites of *Sitona* species produced three species of braconid parasites, none of which occurred in Canada. These were reared from *Sitona* spp., mainly from *S. lineata* (L.), and were identified as *Pygostolus falcatus* Nees, *Perilitus rutilus* (Nees), and a *Microctonus* sp. *Microctonus* sp. is morphologically identical with *M. aethiops* obtained in France; its habits also appear to be similar but there are small differences in the life histories of the two forms. It differs from the French form in being able to develop successfully in *S. cylindricollis* (117).

A large Swedish collection of *S. lineata* in 1958 was 75% parasitized by *P. falcatus* and *P. rutilus*. Parasitism reached a peak at the end of June and the weevil population was drastically reduced by mid-July.

In 1958, 5000 to 7000 *S. cylindricollis* parasitized by *P. falcatus* and *P. rutilus* were released in Manitoba. *P. falcatus* has been recovered for two years, and is probably established.

This project is continuing and it is too early to assess its success. The results of work to date are being prepared for publication by C. C. Loan, Entomology Research Institute for Biological Control, Belleville, Ontario.

Alfalfa Weevil, Hypera postica (Gyll.)

Hypera postica (Gyll.) is a pest of alfalfa in Alberta. In August, 1958, 2000 weevils were exposed to females of *Perilitus rutilus* Nees, a parasite of *Sitona* spp. from Sweden, and then released in the Milk River Valley, Alberta. Less than 12% of the weevils released were parasitized. No parasites have been recovered (118, and Loan, personal communication). This project is still in its infancy, but at this time it must be rated a failure.

Pea Weevil, Bruchus pisorum (L.)

The pea weevil entered Canada at an undetermined date. In the late 19th century it was so important in Ontario that the production of seed peas was almost abandoned. In spite of precautions it became established in British Columbia in the 1920's (120, 206). This insect has received scant attention in the literature and only brief references can be found (62, 65, 66, 119).

The United States Department of Agriculture in 1935 undertook to import natural enemies of the pea weevil. An internal parasite, *Triaspis thoracica* (Curt.), was imported and released in large numbers at widely scattered points in the United States from 1935 to 1942 (40). Some of the parasites were received by the Belleville Laboratory and, in 1942 to 1943, 764 were released near Armstrong, B.C., and 470 in Hastings County, Ontario. No *T. thoracica* were ever recovered, either in the United States or in Canada.

The reasons for the failure of this project are unknown, but some unpublished observations by G. Wishart and W. F. Sellers lead one to believe that the pea weevil is not the natural host of *T. thoracica*. This parasite was obtained from the vicinity of Angern, Austria, where it is so abundant that the relatively small numbers parasitizing the pea weevil could not account for the large population of parasites. The female parasite has great difficulty in piercing the egg of *B. pisorum*, which is laid on the surface of the plant. Other Curculionidae parasitized by *Triaspis* spp. bury their eggs beneath the surface of the plant tissue where they are held firmly while the parasite inserts her ovipositor (Wishart, personal communication).

This project was a failure.

Holly Leaf Miner, Phytomyza ilicis Curt.

Phtomyza ilicis Curt. was introduced to British Columbia and by 1931 was damaging up to 80% of the leaves in commercial holly plantations in the coastal regions. Chemical control was necessary and a project on biological control was launched.

The leaf miner and its parasites were studied in England (39). Five parasitic species were imported: *Epilampsis gemma* (Wlkr.), *E. syma* Wlkr., *Cyrtogaster vulgaris* Wlkr., *Opius ilicis* Nixon, and *Sphegigaster flavicornis* (Wlkr.). These were released on Vancouver Island from 1936 to 1938 and on the lower mainland in 1939. In cage experiments on the Island recoveries were made in 1938 (55) but no imported parasites were found in nature until 1940.

Four of the imported parasites eventually became established but only *E. gemma* and *O. ilicis* became abundant. By 1940, *E. gemma* parasitized up to 80% of the miner in one plantation. A survey in the years 1949–53 (135) showed that *E. gemma* was almost entirely confined to Vancouver Island, where it caused 90% of the total parasitism, and *O. ilicis* was most abundant on the mainland, where it was responsible for 80% to 90% of the total parasitism. This is remarkable because only 10 adults of *O. ilicis* were liberated on the mainland, as compared with 11,000 *E. gemma*. On the Island, 33 *O. ilicis* and 26,000 *E. gemma* were released. In England *O. ilicis* is unimportant and parasitized less than 1% of the leaf miner.

The two uses of holly in western Canada complicate assessment of this project. Holly is used both as a commercial crop for the Christmas florist trade (10,000 lb in 1959) and as an ornamental tree for boulevard, park, and domestic planting. In commercial plantations the introduced parasites do not ensure a blemish-free product. As spraying is still necessary, the project failed. On ornamentals, however, parasitism usually prevents serious disfigurement of the tree and chemical controls are rarely used. From this point of view the project was a success.

A most interesting feature of this project is the establishment of *O. ilicis* on the mainland from a release of only 10 individuals; the size of colony released, therefore, does not necessarily reflect the chances of its success. The fact that in one area one species became dominant and in the other area another did so may support the view that the introduction of many species of natural enemies is advisable. However, one can also speculate that these species compete with one another (*E. gemma* develops early in its host's life

history and prevents the development of mines whereas *O. ilicis* completes its development in the host puparium) in both areas, with different results in each but with a detrimental effect on control in both. Sufficient information is not available to show which is the correct opinion.

Despite the success of the introduced parasite complex in controlling damage on ornamental trees, the fact that it failed to produce economic control in orchards prevents us from rating the project as a full success. As a measurable reduction of damage to holly trees was produced, however, we are justified in calling it a success in part.

Cabbage Maggots, Hylemya spp.

Cruciferous crops in Canada have for a long time been subject to damage by root maggots. Over the past 75 years much has been written on the occurrence, life history, and control of "the cabbage maggot", *Hylemya brassicae* (Bouché), or species that have been misidentified as this. Brooks (26) collected from Cruciferae all over Canada and found dipterous species of several different families in the roots. Three species, all of the genus *Hylemya*, attacked living roots and caused primary damage: *H. brassicae* (Bouché), *H. floralis* (Fall.) (=*H. cruciferae* Huck.), and *H. planipalpis* (Stein).

Wishart (220) studied the parasites of *Hylemya* in Canada from 1951 to 1953 to assess the possibilities of biological control of root maggots. He examined four species of *Hylemya*, the three mentioned above and *H. cilicrura* (Rond.). Five parasite species were found, two of which, *Aleochara bilineata* Gyll. (Staphylinidae) and *Trybliographa rapae* (Westw.) (Cynipidae), were distributed throughout Canada on all four host species; two, *Phygadeuon* sp. (Ichneumonidae) and *Aphaereta pallipes* (Say) (Braconidae), were rare and probably parasitized *Hylemya* spp. only incidentally, and the remaining one, *Aleochara bipustulata* (L.), was present in small numbers in several areas. Prior to Wishart's study several authors had recorded parasites, mostly staphylinids and cynipids, from *Hylemya* species (67, 73, 163). Some of the species reported were misidentified, and others proved to be synonyms of one or other of the above species. Thus there are only three species of parasites of any importance on *Hylemya* spp. in Canada.

Wishart showed that parasitism by these three species was variable: in *H. brassicae* it ranged from 1.2% to 87.2%, with an average of about 29%; in *H. cilicrura* from 2.6% to 100%, with an average of about 27%; in *H. planipalpis* the average was about 21.4%; and in *H. floralis* parasitism ranged from 1.2% to 33.3%, averaging around 13%.

The parasites of *Hylemya* spp. were studied in Europe at the same time as in Canada (219). The European situation was virtually identical with that in Canada; the same species of *Hylemya* were dominant, and they were attacked to the same degree by the same parasites. It was therefore concluded that parasite introductions from Europe would be futile, and the project was dropped.

This study demonstrates an intelligent approach to biological control. Earlier, because of confusion in the taxonomy of both hosts and parasites, it was assumed that the species of both differed in Europe and Canada. Some parasites were introduced to Canada on the basis of this belief. This study stim-

ulated the necessary taxonomic work that exposed the fallacy of this premise, and importations were immediately abandoned.

As a control project this work obviously failed, but as a scientific exercise it was most laudable.

Carrot Rust Fly, Psila rosae (F.)

Psila rosae was recorded in Canada in 1885 (126) and now is a serious pest of carrots in many areas, particularly in British Columbia and Ontario. An attempt to control it by importing parasites was made in the 1940's but the results were never fully published. Two species of parasites, *Dacnusa gracilis* (Nees) and *Loxotropa tritoma* (Thoms.), that attack the fly in England (222) were introduced and released, both in Ontario and in British Columbia, about 10 years ago. McLeod (in preparation) reported that neither species was ever recovered from field-collected rust fly in British Columbia, and Maybee (126) reported that a few specimens of both were recovered the year following release in Ontario but never in succeeding years. The attempt was a failure.

European Wheat Stem Sawfly, Cephus pygmaeus (L.)

Cephus pygmaeus (L.) was introduced to Canada from Europe at an early date and first recorded in Ontario in 1887 (94). Early surveys indicated that the sawfly was controlled by two native parasites, *Heterospilus cephi* Rohw. and *Pediobius beneficus* (Gahan) (173). In 1938, however, the sawfly damaged wheat in several areas and from 1937 to 1940 releases of *Collyria calcitrator* (Grav.), a parasite of *C. pygmaeus* from Europe, were made in Ontario. Annual collections of sawfly cocoons from wheat stubble were made until 1950. Parasitism by *C. calcitrator* increased steadily from 1% to 2% in 1939 to about 20% in 1945. It was not the most abundant parasite in this period but was second to *P. beneficus*. Parasitism by all species varied from 5% in 1939 to 35% in 1946. From 1946 to 1950 a marked increase in parasitism by *C. calcitrator* accompanied the decline of host numbers and *C. calcitrator* ultimately became the most abundant species attacking the sawfly. Annual collections of sawfly cocoons were discontinued in 1950 because of scarcity. Occasional collections in 1950–57 showed that *C. calcitrator* was present at even extremely low host densities. In 1958 the sawfly occurred in no more than 1% of the wheat stems in any field sampled and *C. calcitrator* was always found with it. We therefore agree with Smith (174) that the introduction of *C. calcitrator* tipped the balance against *C. pygmaeus* in Ontario. Before *C. calcitrator* was introduced the sawfly apparently was becoming a serious pest. After its introduction sawfly populations declined to insignificance and have remained there. The project was successful.

Western Wheat Stem Sawfly, Cephus cinctus Nort.

Cephus cinctus Nort. is a native pest of wheat in western Canada and has been well documented (46, 149). Briefly, this sawfly originally fed on grasses of the western plains and became a pest when large areas were planted to wheat around the turn of the century. A number of native parasites attack the sawfly on native plants but have not adapted to cultivated conditions (47, 162). In the late 1920's the Imperial Bureau of Entomology investigated parasitism of several *Cephus* species in Europe.

Salt (161) recommended the importation of *Collyria calcitrator* (Grav.), a parasite of *C. pygmaeus* in Europe. Life tables prepared for *C. pygmaeus* showed that the effect of *C. calcitrator* superimposed on those of relatively constant harvesting, fall ploughing, and winter mortality controlled the sawfly in England.

In 1930 about six thousand *C. calcitrator* from England were released in Saskatchewan, and in the following 10 years about 450,000 were released on the Canadian prairies. They were observed parasitizing *C. cinctus* in wheat stems, and autumn collections at the release point showed parasitism of from 2% to 9%. Some colonies survived for two years and then disappeared (171, and Smith, personal communication). At this late date it is difficult to assess the reasons for this failure. There is, however, a difference in the habits of *C. pygmaeus*, the usual host of *C. calcitrator*, and *C. cinctus* that may be at least partly responsible for the failure. *C. calcitrator* is an egg parasite. In England, its host *C. pygmaeus* usually lays a single egg per wheat stem and if this egg is parasitized the host is killed and an adult parasite emerges. *C. cinctus*, however, may lay as many as 12 eggs per stem, only one of which ever survives; the others are eliminated by competition. To be certain of killing the survivor of the competition, therefore, all 12 eggs must be parasitized, and at least six must be parasitized to provide a 50–50 chance. Furthermore, if parasitized sawflies are at any competitive disadvantage in relation to unparasitized sawflies, all eggs must be parasitized before there is any chance of destroying the winner of the competition. It is unlikely that all the eggs in a stem should be parasitized and this may account for the failure of *C. calcitrator* in western Canada.

The project failed.

Spruce Sawfly, Diprion hercyniae (Htg.)

Diprion hercyniae (Htg.) was introduced to North America from Europe before 1920. It was mistaken at first for the common European species, *Diprion polytomum* (Htg.), but differences in ecology, cytology, and morphology revealed that the Canadian form was a separate species subsequently found to be endemic in Europe (12, 175, 176). In 1930, the first serious outbreak caused noticeable defoliation of spruce over an area of 2000 square miles on the Gaspé Peninsula. By 1937 about 10,000 square miles were heavily infested and many trees were killed. Moreover, the insect was widely distributed throughout most of the spruce forests of eastern North America and was "generally the most abundant species feeding on spruce in the region" (11).

The life-history of *D. hercyniae* and the damage it causes were fully described by Balch (11). In eastern Canada predation and parasitism were moderate (17, 95, 156) except that small mammals destroyed up to 50% of the cocoons (140). In some areas the only effective regulating factor was the local exhaustion of food (13).

Parasite importations were started in 1934. Though 27 parasite species were introduced from Europe, only five species became established on *D. hercyniae* in North America: *Drino bohemica* Mesn., and four species of *Exenterus—E. tricolor* Rom., *E. confusus* Kerr., *E. vellicatus* Cush., and *E. amictorius* (Panz.). An additional parasite, *Dahlbominus fuscipennis* (Zett.), is widely reported in

the literature to be established on *D. hercyniae*. Almost 900 million individuals of this species were released and though recoveries from *D. hercyniae* were frequent before 1945, none have been recovered from *D. hercyniae* since that time.

A virus disease of the sawfly was observed in the forest in 1938 and became abundant in parts of New Brunswick, Vermont, and New Hampshire by the end of that year (13, 17, 54). By 1942 it was distributed over the entire range of the sawfly. Before the disease appeared other mortality factors, including the two introduced parasites *D. fuscipennis* and *E. confusus*, accounted for about 85–95% of each generation. The virus forced mortality well above 98% and reduced the population so drastically that all other regulating agents became unimportant. The disease continued to dominate until 1942, after which it lost its effectiveness and the sawfly population rose moderately. Disease struck again in 1945 and 1946 and both the sawfly and its disease were reduced to very low levels. This situation prevailed until 1951. Small annual collections during these and later years showed that the disease persisted even at very low host populations (17). The origin of the disease is unknown but it is thought to have been introduced into North America on parasite material (13).

A moderate increase in the sawfly population occurred in 1951 and 1952, but for unknown reasons a corresponding increase in the disease did not follow. *D. fuscipennis* and *E. confusus* were apparently eliminated as effective regulating agents following the collapse of host populations (157). After 1944, however, *D. bohemica* and *E. vellicatus* became increasingly effective. They contributed to the decline of *D. hercyniae* in 1945, were mainly responsible for keeping it down from 1946 to 1951 (17), and probably controlled the outbreak of 1951. They reached the peak of their effectiveness in 1953 when *D. bohemica* accounted for 58.5%, *E. vellicatus* for 9.2%, and disease for 7.8% of the sawfly larvae, while *D. bohemica* killed 34.1% and *Exenterus* sp. (*vellicatus*?), 19.2% of the cocoons.

Of the five (or six) parasites established on *D. hercyniae* in North America only *D. bohemica* and *E. vellicatus* are of any significance in population regulation. Of these, *D. bohemica* is the most important.

This project is an outstanding success for biological control even though the foremost agent of control was introduced by chance.

European Pine Sawfly, Neodiprion sertifer (Geoff.)

N. sertifer is a native of Europe, where it has a long history of population eruptions and severe damage to pine forests. Its discovery in Canada in 1939 therefore caused considerable concern. It now ranges throughout southwestern and south-central Ontario. The eastward and northward spread of the sawfly prompted an attempt to control it by introduced parasites, and 11 species originally acquired for release against *D. hercyniae* were used. Most of these parasites were obtained from eggs and cocoons of *N. sertifer* in Europe.

Establishment of parasites in Canada has been disappointing. *Dahlbominus fuscipennis* (Zett.) has apparently become firmly established on this host but has failed to spread with the host or to achieve an effective density. *Exenterus amictorius* (Panz.) failed to become established on *N. sertifer* until the range of

this host overlapped those of *D. hercyniae* and *D. similis* in the area of Galt, Ontario; since then *E. amictorius* has been recovered from *N. sertifer*. Though egg parasites are common on *N. sertifer* in Europe, three species introduced to Canada have failed to become established. The failure of two of the species may have been because inadequate numbers of parasites were released. Synchronization of egg parasites with their host development appears to be critical, and improper timing of releases may also have been responsible for failure. Further efforts are being made to overcome problems of release and synchronization and the project is still active.

Finlayson and Finlayson (63) and Griffiths (90) have shown that the sawfly is attacked by a complex of indigenous species as well as by those imported species that have become established.

Bird (16) discussed the use of a virus disease brought to this country from Sweden to control *N. sertifer*. This seems to have been partially successful but population levels, though reduced, are still undesirably high. It is hoped, therefore, that the addition of one or two more successful mortality agents may reduce this level to a satisfactory point. Rather than rate this attempt as a complete failure, we have judged it "without success to date".

Larch Sawfly, Pristiphora erichsonii (Htg.)

The first identification of *P. erichsonii* on this continent was by Hagen in 1881 (92), but there is a history of larch defoliation, probably by *P. erichsonii*, dating back to the beginning of the 19th century. The species is apparently native to Europe.

Fletcher (68) described an outbreak of *P. erichsonii* that started in 1882 and destroyed "millions of acres" of larch in eastern Canada. Since then many records of larch sawfly outbreaks have appeared in the literature (48, 97, 101, 111, 158, 196). After 1882 the sawfly has moved steadily westward until about 1942 it reached the western limit of larch in Canada, near the Okanagan Valley of British Columbia.

Natural regulating factors include winter flooding of larch sites (103, 111, 112, 113, 114, 115), predators, mainly small mammals (34, 35, 89, 141), limitation of food supply, and parasites.

Mesoleius tenthredinis Morley, an introduced ichneumonid, is the principal parasite of *P. erichsonii* over most of its range. In 1910 and 1911, cocoons parasitized by *M. tenthredinis* were acquired from England and released in Ontario. These early introductions failed, but later releases in Quebec and Manitoba were highly successful (100). The first record of establishment was from Treesbank, Manitoba, in 1916 (48). By 1920 a parasitism of 66% was recorded and the sawfly population in that area collapsed. A scarcity of hosts prevented further records until 1926, when a parasitism of 66% was again recorded. This population was used as a source of *M. tenthredinis* for redistribution over large areas of Manitoba and Ontario. In 1935, *M. tenthredinis* was well established in Quebec and was distributed from there throughout Ontario and the Maritime Provinces (83, 85). The parasite was firmly established in the Maritimes by 1937 (158).

M. tenthredinis was introduced to British Columbia in 1934. As *P. erichsonii* spread rapidly to the north and west, parasites were released in the newly

invaded areas. In each area, *P. erichsonii* populations became heavily parasitized and subsided without causing serious tree injury (134).

The initial success of *M. tenthredinis* in the prairie provinces was not sustained. About 1940 it was noted that, though a large percentage of sawfly larvae were parasitized, most of the parasite eggs failed to develop. Unhatched eggs were found to be "encapsulated" within the host, and embryonic development ceased in three to four days. Encapsulation occurred in both Manitoba and British Columbia but, whereas in Manitoba over 95% of eggs were thus affected, in British Columbia less than 5% were encapsulated. Thus, in Manitoba, and elsewhere in central North America, *M. tenthredinis* became ineffective as a control of *P. erichsonii* and sawfly populations again rose (143, 144). In the rest of Canada, *M. tenthredinis* remains an important control factor.

Two other parasites contribute to the control of *P. erichsonii*: the tachinid *Bessa harveyi* (Tnsd.) (104) and the native chalcid *Tritneptis klugii* (Ratz.) (101). The origins of *B. harveyi* are obscure. Apparently identical forms occur in Europe and North America. It is now the most important parasite of *P. erichsonii* in regions where *M. tenthredinis* is encapsulated.

In spite of the failure of *M. tenthredinis* in the mid-western regions, this parasite regulates *P. erichsonii* at a satisfactory level of abundance in large areas of eastern Canada and in the far west. We therefore rate the project as a success.

Discussion

Of the 31 projects reviewed here we have rated 12 as successful, one (holly leaf miner) as successful in part, i.e., successful for one economic product but not for another, two (pea moth and the Oriental fruit moth) as partially successful, i.e., causing an appreciable reduction of the host without achieving control, and three (sweetclover weevil, alfalfa weevil, and European pine sawfly) as without success to date, i.e., the projects are continuing with prospects of eventual success. The remaining projects were failures. Thus, even if the 29 incompletely documented cases are eliminated, well over half the projects have failed to achieve a satisfactory control. This record cannot be considered good and indicates a need to revise methods. In the following section we will outline what we think is a sound approach to biological control projects, pointing out deficiencies of past projects and where, in our opinion, fresh thinking is needed.

Suitability of Biological Control

The first point to be considered in any control project is the means by which control is most likely to be achieved. This requires a consideration of the use to which the crop is to be put, the economic conditions under which it will be marketed, and the type of damage inflicted by the pest. In the apple-growing industry for example, present market standards demand virtually perfect fruits. The apple codling moth is almost a universal pest of apples, and, when uncontrolled, often completely destroys the crop. Biological agents could conceivably reduce the damage to fractional levels and ensure the production of a crop. But a single moth larva attacking an apple will render

that apple valueless, and a loss of from 4% to 5% of apples in a crop often eliminates the grower's margin of profit. To achieve a satisfactory control, therefore, codling moth numbers must be reduced to fewer than one larva for each 20 apples on the tree. It is doubtful whether the most efficient of biological agents could maintain the moth population at this level.

On the other hand, the spruce sawfly is an important pest of Canadian forests. This insect destroys the foliage of spruce trees, causing loss of growth, deterioration of vigor, and even, if the defoliation is sufficiently severe and protracted, death of the trees. But the wood, which is the principal product at stake, is not immediately or directly destroyed. Moreover, extensive damage to the foliage may continue for a considerable period without causing intolerable economic loss. Efficient biological agents are able to reduce populations of spruce sawfly to levels where damage to the foliage, though persisting, is of little economic consequence.

These examples illustrate an important feature of insect pest problems. Certain pests, relatively small populations of which by directly attacking produce immediately destroy a significant part of its value, we designate as direct pests; other pests, which we call indirect pests, attack produce and cause economically significant damage only by intensive or extended infestations. The main difference between these categories lies in the number of individuals that can be tolerated in the environment; in the case of indirect pests, moderately high populations may be tolerated; in the case of direct pests numbers must be reduced to the verge of extinction. Indirect pests are suitable subjects for biological control; direct pests are not.

We feel that this is a principle of universal application in spite of apparent successes of biological agents in the control of fruit pests in California. One of these that was recently brought to our attention is the control of the olive scale, *Parlatoria oleae* (Colvee), by the introduced parasite *Aphytis maculicornis* (Masi) (51, 52, 102). These authors do not supply enough information to allow us to estimate the number of scales that are required to make an olive unmarketable; thus we cannot guess at the intensity of attack that can be tolerated. We note, however, that the residual population at the "commercial control" level remains moderately high, part of it feeding on twigs where the scales apparently do no significant damage, and part of it on the fruits where they cause up to 4% cull. A direct pest, the codling moth, for example, with a residual population of this magnitude probably would cause a much higher proportion of culled fruit. On the basis of this rather scanty evidence we feel that the olive scale is probably an indirect pest.

Categorizing any biological phenomenon in terms of black or white always presents difficulties, and we have found such difficulties in categorizing the pests involved in Canadian biological control projects into our two classes. There are marginal cases that could conceivably fall into either class. However, after careful consideration we have classified each of the 31 pests examined as either direct or indirect pests. For the purposes of this tabulation we have rated the partial successes as failures. Some pests we have been compelled to place in both categories; the holly leaf miner, when it attacks holly foliage in orchards, is a direct pest and is uncontrolled, but when it attacks ornamental trees it is an indirect pest and is controlled; the European corn borer when it

TABLE I

Canadian insect pests against which determined biological control measures were taken, classified into "direct" and "indirect" pests, with a record of the success (S.) or failure (F.) of the control achieved in each case

Direct pests	S.	F.
European corn borer—in ears *Ostrinia nubilalis* (Hbn.)		X
Pea moth *Laspeyresia nigricana* Steph.	Partial success	
Oriental fruit moth *Grapholitha molesta* (Busck)	Partial success	
Balsam woolly aphid *Adelges piceae* (Ratz.)	?*	X
Cabbage maggots *Hylemya* spp.		X
Pea weevil *Bruchus pisorum* L.		X
Carrot rust fly *Psila rosae* F.		X
Holly leaf miner—orchards *Phytomyza ilicis* (Curt.)		X
Codling moth *Carpocapsae pomonella* (L.)		X
Good control	0	
Partial control	2	
Failures	7	

Indirect pests	S.	F.
European corn borer—in stems *Ostrinia nubilalis* (Hbn.)		X
European wheat stem sawfly *Cephus pygmaeus* (L.)	X	
Western wheat stem sawfly *Cephus cinctus* Nort.		X
Apple mealybug *Phenacoccus aceris* Sig.	X	
Woolly apple aphis *Eriosoma lanigerum* (Hausm.)	X	
European earwig *Forficula auricularia* L.	X	
Pine shoot moth *Rhyacionia buoliana* (Schiff.)		X
Holly leaf miner—ornamentals *Phytomyza ilicis* (Curt.)	X	
Spruce budworm *Choristoneura fumiferana* (Clem.)		X
European pine sawfly *Neodiprion sertifer* (Geoff.)		X
Spruce sawfly *Diprion hercyniae* (Htg.)	X	
Larch casebearer *Coleophora laricella* (Hbn.)	X	
Lodgepole needle miner *Recurvaria starki* Free.		X
Satin moth *Stilpnotia salicis* (L.)	X	
Oystershell scale *Lepidosaphes ulmi* (L.)	X	
Grasshoppers		X
Greenhouse whitefly *Trialeuroides vaporariorum* Westw.	X	
Citrus mealybug *Pseudococcus citri* Risso	X	
Grape mealybug *Pseudococcus maritimus* Ehrh.		X
Sweetclover weevil *Sitona cylindricollis* Fahr.	?*	X
Alfalfa weevil *Hypera postica* Gyll.	?*	X
Brown-tail moth *Nygmia phaeorrhoea* (Donavan.)		X
European fruit lecanium *Eulecanium coryli* (L.)	X	
Larch sawfly *Pristiphora erichsonii* (Htg.)	X	
Good control	13	
Failures	11	

*Projects are continuing with some chance of ultimate success.

attacks corn intended as use for fodder is an indirect pest, but when it attacks ears of corn grown for grain it is a direct pest. By splitting each of these projects into two parts we have, for the purpose of this tabulation, raised our total number of projects to 33. Nine of these were against direct pests, none of which were successfully controlled, and 24 were against indirect pests, 13 of which were successfully controlled.

Lloyd (116) suggested that biological agents have been successful mainly "where the host plants are trees or other perennials maintaining their geographical locations over periods of many years." He attributes such successes to the stability of the environment induced by perennial plants. Testing this hypothesis against Canadian experience we find that we have had more successes against pests of perennial plants than against those of annuals or

biennials. On the basis of 33 projects (counting the corn borer and holly leaf miner each as two projects), we have conducted 15 operations against pests of annual or biennial plants, and 18 against pests of perennial plants, mainly trees. Against annuals and biennials we have had four successes, against perennials nine. All our successes, however, regardless of the longevity of the plant or the stability of the environment, were against indirect pests. What truth there may be in Lloyd's conjecture regarding type of host plant we therefore attribute to the degree of damage the crop can tolerate rather than to environmental stability.

In general, perennials can withstand certain types of damage, such as defoliation, much better than can annuals, and the resulting loss of production is of less economic consequence to perennials than to annuals. But if the product of a perennial, such as apples or peaches, is directly attacked by only a small population of their respective pests, the economic consequences are quite as serious as similar damage to annual plants, and stability of the environment induced by the perennial habit of the orchard does nothing to reduce this vulnerability to damage. In any event, we are unconvinced that perennial plants necessarily contribute more stability than annuals to some environments. What plant community is more stable than the wheat fields of the Canadian prairies? Here identical crops pass through identical cycles year after year for generations. Crop rotation practices never move a crop further than a few hundred yards, and there is usually a stand of wheat within easy flying distance of most parasites no matter in which direction they may fly. The same situation applies to many other regions where certain crops are grown intensively. Trees, on the other hand, and especially forests, though they may remain in the same spot for years, develop progressively from year to year and no two years are identical. The changes from one year to the next may be small, but in 10 years obvious changes occur. The much-vaunted stability of forest environments is probably more apparent than real.

We therefore reject Lloyd's hypothesis and prefer to use the type of damage inflicted by an insect, the amount of such damage that can be tolerated, and the pest population density required to produce intolerable damage as our criteria of the suitability of biological agents to solve a given pest problem. The relative number of successful biological control operations against pests of perennial plants as opposed to pests of annual plants is a product of these factors rather than the result of any inherent qualities of the perennial habit of plants.

In this review we have kept in mind certain matters regarding the general suitability of biological control that have been suggested by other authors. Our findings, or rather our lack of findings, on these matters were disappointing.

On the positive side was the light that our experiences shed on Taylor's suggestion (199) that biological control is most effective on islands and in other well-defined and limited areas. In some of the successful Canadian projects support can be found for this hypothesis, particularly in the projects conducted on the coastal plain of British Columbia and in greenhouses. In others, however, where control was successful over large continental areas, both east and west, no support can be found. As a dictum, then, the "island concept" is invalid, though as a generality it may be true that the chances of

successful biological controls in well-defined, limited areas are greater than in other situations.

We could find no striking correlation between the habits of an insect and its vulnerability to biological control, though our sample was admittedly small. Insects that spend a long time in what seems to be a well-protected situation are apparently neither more nor less immune to biological agents than those that seem to invite attack at most times. In instances where a correlation was suspected, other factors were concluded to override the importance of the biological factors. For instance, though scale insects seem to be particularly vulnerable to biological control, their obvious exposure to parasitism and predation is thought to be a less important feature of their control than their status as indirect pests. The Oriental fruit moth, on the other hand, burrows deep into the fruit, where it is well protected from its enemies. Despite this habit the fruit moth is heavily parasitized, and it is considered uncontrolled only because of the direct nature of the damage inflicted by it.

We could conclude little from our data about the relative importance of mono- and poly-cultures in biological control. Biological controls are applied only where organisms have become pests and, therefore, only to situations where insects are competing with humans. In Canadian experience most of these situations occurred in monocultures and there were not enough experiences involving polycultures for conclusions to be drawn. Once again our sample is too small.

Selection of Biological Control Agents

If a pest and its ecology were thoroughly understood it should be possible to define precisely the attributes of the biological agent that would be most effective against it. We could then preselect for introduction only those organisms that closely reflect this ideal, and we should be able to predict accurately the probable success of each agent in the new environment. Unfortunately our present knowledge is inadequate for any such preselection or prediction. Most of our efforts to do so have failed. Thompson (200) claimed that environments are so complex that it will always be impractical to consider all the factors that may influence the success of an organism. Moreover, since predictions could only be confirmed by actually introducing the organisms into the new environment, Thompson concludes that we may as well introduce organisms in the first place and determine their suitability by their performance in the new environment. This philosophy seems to us to abandon all hope of placing biological control on a scientific basis. It is analogous to a medical doctor who, when confronted with an ailing patient, doses him with a random selection of medicines in the hope that none will harm him and one might possibly cure him.

We agree that difficulties confront all efforts to predict which organisms will be successful in a new environment. But we suggest that such predictions should be made to the best of our ability and only carefully preselected organisms should be introduced. The actual performances of the selected organisms in the new environment can be checked against the predicted performance with the objective of improving predictions.

In practice, of course, organisms for introduction have always been preselected, and this has always been based on a prediction of probable success

of the organism in the new environment. For instance, when parasites were wanted to control the spruce sawfly, only parasites of the same or closely related host species in Europe were introduced because such parasites were considered more likely to succeed on *D. hercyniae* in Canada than others that were available. Many of the parasites chosen on this basis failed to become established in Canada, but at least two fully lived up to the prediction. Again, when biological agents were needed to control the holly leaf miner in British Columbia, several parasites of the same host in Britain were introduced in preference to parasites of other hosts. *Epilampsis gemma* was given priority because it was the most successful parasite of *P. ilicis* in its native environment, and it was therefore predicted that it would have the best chance of success in Canada. This prediction proved only partly correct: *E. gemma* succeeded as expected on Vancouver Island but failed on the mainland for unknown reasons.

The relative success of a parasite species against a host in its native environment has seemed a reasonable basis for predicting its success on the same host in a new locality. In practice this led to false hopes in a remarkable number of instances. There are many small differences between even superficially similar environments, any one of which may cause a species to succeed in one and fail in another. Therefore a parasite that plays an insignificant role in its native locality may succeed beyond expectations in a new area. It may, for example, be freed from the interference of a competitor better adapted than itself to the native environment, but less well adapted to the new one. This may have been the case with *Opius ilicis*, a rare parasite of the holly leaf miner in England. Ten adults of this species were released on the British Columbia mainland, and 86 on Vancouver Island. In the presence of the successful *E. gemma* on Vancouver Island, as in its native England, *O. ilicis* failed. But on the mainland, where *E. gemma* failed, the minute colony of *O. ilicis* flourished and the species became the dominant parasite of the miner in that area.

Climatic considerations are a further basis for the preselection of biotic agents of control. Most of the agents that have been introduced into Canada originated in Central Europe or England. But it has become apparent that many of these agents are incapable of adjusting to the rigorous Canadian winters. There is therefore a growing tendency to obtain agents from more-northerly areas where climatic conditions are more similar to those of Canada. Efforts are being made to establish contacts with eastern European countries from which we have been barred for political reasons. This will give us an opportunity to select agents that will have a better chance of succeeding in our climate than the ones now available to us.

These criteria for preselection of biotic agents are undeniably crude and the long list of attempted introductions that have failed indicates their inadequacy. But if we cannot yet define the sort of organisms that will control a particular pest, we can at least learn from past errors to avoid those that have virtually no chance of success.

No rational selection of biotic organisms can be contemplated without adequate knowledge of the ecological situation at both ends of a potential movement. Ideally, studies in both areas should be conducted by the same persons, but if this is impossible close liaison between researchers in the two areas should be established. Failure to obtain and correlate adequate information on

the identities, biologies, and distributions of hosts and parasites, and the relative ecological situations prevailing in the two areas, has resulted in much waste of time and effort.

The identification of host and parasite species is one of the most basic and most difficult tasks. It has delayed the successful conclusion of many projects and has led others down unproductive paths. *Diprion hercyniae*, the common spruce sawfly, was for several years confused with its near relative *D. polytomum*, though the two have different reproductive methods and habits. *Adelges piceae*, the balsam woolly aphis, was for many years confused with *A. musslini*, a species that is attacked by a different complex of predators; this confusion also confounded analyses of the damage caused by *A. piceae*. *Evagora starki*, the lodgepole pine needle miner of Canada, was confused with *E. milleri* and a number of other needle miners, resulting in attempts to introduce several unsuitable parasites. Perhaps the outstanding case of confused identities concerns the cabbage root maggot project. When errors of nomenclature were corrected it was apparent that all the known parasites of the European root maggots were already in Canada and that further importations would be futile.

No less important is a thorough knowledge of the habits, life-histories, and distributions of the species involved. The lack of, or disregard for, such knowledge has led to many abortive introduction attempts. For instance, critical differences between the egg-laying habits of the two wheat stem sawflies in Canada suggest that the probability of controlling *Cephus cinctus* by *Collyria calcitrator*, a parasite of *C. pygmaeus*, was at best small. Some 27 parasite species were released in Canada against the European spruce sawfly, *Diprion hercyniae*. The primary host of most of these was *Neodiprion sertifer*. Some, including *Dahlbominus fuscipennis*, which was released by the hundreds of millions, were never found parasitizing *D. hercyniae* or its close relative *D. polytomum* in their native environments though they were common in the range of these hosts (138, 139). The life history of *N. sertifer* differs markedly from that of *D. hercyniae* in that the former normally overwinters in the egg stage and the latter as a cocooned larva in the soil. The fact that *D. fuscipennis* was recovered from *D. hercyniae* for several years was taken as evidence of its establishment on this host, but it is significant that these recoveries ceased almost simultaneously with the cessation of releases of the parasite.

An illustration of the limits imposed by poor synchronization of host and parasite may be found in the European corn borer program. For many years parasites were imported into Canada from multivoltine strains of the host in Europe. The Canadian strain was mainly univoltine, with a few multivoltine individuals. The introduced parasites met with little success. But recently the multivoltine strain in the Canadian population has been gaining ascendancy, and this has been accompanied by an increasing success of some species of introduced parasites. This suggests that the initial failure of the parasites was caused by poor synchronization between the parasites and the univoltine host, a weakness that is becoming less important with the development of a multivoltine host population.

The question of variation within a parasite species is rarely given adequate attention. There are at least two instances in Canadian experience when a

strain of a parasite from one region failed whereas another strain of the same species from a different region succeeded promptly. The parasite *Bigonicheta spinipennis* from earwigs collected in England failed to become established on this host in British Columbia, though smaller colonies of the same parasite from the south of France achieved almost immediate success. Repeated attempts to establish *Microctonus aethiops* from France on the sweetclover weevil in Manitoba failed, but a strain of the same species from Sweden readily became established. A further case of what may be two distinct strains of one species involves the braconids known as *Ascogaster carpocapsae* (Vier.) and *A. quadridentatus* Wesm. The two entities are morphologically identical, and the former name is now considered a synonym of the latter. But *A. quadridentatus* is a parasite of the pea moth and *A. carpocapsae* of the codling moth; the two hosts apparently are not interchangeable.

Hosts too can have a multiplicity of strains, and parasites that fail on one strain may succeed on another. The corn borer situation, where parasites were less successful on the univoltine strain than on the multivoltine strain, has been mentioned. The larch sawfly and its parasite, *Mesoleius tenthredinis*, is another example. *M. tenthredinis* is successful on strains of the host that do not encapsulate the parasite egg, but is ineffective in central and mid-western areas where an encapsulating strain of the host has developed.

The number of parasite introductions that failed because there was not a thorough search for appropriate strains can only be guessed; similarly the number that were unsuccessful because parasites were not released against the full range of host variation is unknown. This is a matter that is receiving more consideration than in the past.

There is little point in trying to establish a parasite or a predator on a host species that it cannot find or will not accept. Little is known of the characteristics of a host that make it suitable in these ways, though presumably they include, as well as those of the host itself, the characteristics of its environment. Nevertheless, some opinion on this question should be formed and organisms that are unlikely to find or accept a particular host should be rejected. This principle seems to have been ignored on many occasions: the predator *Lipoleucopis praecox* de Meij. (Diptera: Chamaenyiidae) was imported to attack *Adelges piceae* although it is probably a specific predator of *Pineus pini* (L.); *Rhizophagus* sp. (Coleoptera: Rhizophagidae) and *Rhopalicus tutela* (Walk.) (Hymenoptera: Pteromalidae), introduced to combat the eastern spruce bark beetle, *Dendoctronus piceaperda* Hopk., failed to become established on this host, probably because these parasites normally attack bark beetles on pine and are not attracted to spruce; *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae) was released in Canada against the winter moth, *Operophtera brumata* (L.), a defoliator of deciduous trees, though this parasite is usually confined to hosts on coniferous trees; *Ascogaster quadridentatus* (Wesm.) and *Macrocentrus ancylivorus* Rohw. were released in Canada against the spruce seed moth, *Laspeyresia youngana* (Kearf.), though their natural hosts are confined to angiosperms; *Agrothereutes abbreviator* Fab. (Hymenoptera: Ichneumonidae) was introduced to Canada from Europe to attack the European pine sawfly, *Neodiprion sertifer* (Geoff.), though in its native environment this parasite mainly attacks various Lepidoptera and only

occasionally attacks sawflies, mainly *G. hercyniae* on spruce; *Melanichneuman mucronatus* (Provancher) (Hymenoptera: Ichneumonidae) was released against the European corn borer though it was known to parasitize that host only in captivity and in the absence of more-attractive hosts. Many more cases could be cited. It is true that many of these organisms were either surplus stocks imported for more-suitable hosts, or were acquired incidentally in operations designed to secure more-suitable organisms, and were released in the hope that they might do some good and were unlikely to do much harm. We will discuss the validity of this assumption in a later section.

These are but some features of particular biotic agents that experience has shown made them unsuitable for introduction against particular hosts under particular circumstances. They are examples of the sort of thing to look for in assessing the probable success of a potential biological control agent. They do not assist us much in predicting what agents will succeed in any specific case, but they do help us in deciding what agents are unlikely to succeed and should be rejected. Nearly every well-conducted biological control project will reveal some such lesson and, if for no other reason than this, each situation in which biological control is attempted should be thoroughly studied before, during, and after the operation so that lessons such as these may be observed and recorded. In many past projects, such studies were not made, or if they were made the observations were not recorded. Thus we can learn nothing from them.

Another point about the selection of biotic agents is the number of species of organisms that should be introduced to attack a single pest. If a single efficient species produces an adequate control, will the addition of 10 more species improve the control or make it more certain? If there exists an emergency pest situation, should we immediately introduce a large number of species, at least some of which *might* alleviate the situation, or should we delay introductions until we find one species that promises complete success? If we discover a number of species all with apparently equal chances of success, should we introduce them all simultaneously? In the case of an introduced pest, should we try to duplicate in Canada the entire complex that regulates it in its native home, or should we restrict our selection to one or a few highly efficient agents?

These are critical questions to persons involved in economic entomology. Unfortunately there are no clear answers. Our usual practice is to introduce as quickly as possible all the organisms that attack the pest or any relative of the pest in any part of the world. By and large, the method has proved practical, and there is no way of showing that it has ever caused any significant harm. But we rarely know why one species succeeded while another failed; in the end we find we have not advanced our knowledge or skills any appreciable amount and must start our next project from the same base-line. Thompson (200) said "the introduction of a beneficial insect into new countries . . . constitutes, in a certain sense, a new and unrepeatable experiment". As usually conducted, such "experiments" are very badly designed; they usually conceal more information than they reveal. And they are unrepeatable; what we do not learn from the first experiment we will never learn. When we introduce several species into a new environment simultaneously we lose forever the

opportunity of learning how each would react to the new environment in the absence of the other, i.e., in the absence of competitors.

It is generally conceded that the best subjects for biological control are organisms accidentally introduced into a new region without the large complex of parasites and predators that attack them in their native homes. These organisms may be slow to attract parasites and predators in the new region; they often find large and virtually unexploited sources of food and are, therefore, able to expand their numbers enormously, uninhibited by potent biotic agents of mortality. Compared with the complex interrelations existing between an animal and its environment when the two have evolved together for countless generations these immigrant animals present a relatively simple problem; their relations with their environment are incompletely established and are amenable to manipulation. Very often the introduction of one new biotic agent will limit their numbers to a satisfactory level. Such newly introduced agents themselves assume a position in the new environment similar to that occupied by their host, and factors identical with those that ensured the success of the host will operate for them also: they are introduced into a new region without biotic agents to attack them; they find an abundance of food in the form of an epizootic host population; there are few other organisms utilizing the same food source to compete with them; they are free to expand their numbers to the limit of their food supply, i.e., their host. They eventually overexpand and deplete their food supply and thus bring about control of the pest.

We can point to a number of examples where such a chain of events occurred in Canada: for instance, the control of the lecanium scale by *Blastothrix sericea*, the woolly apple aphis by *Aphelinus mali*, and the apple mealy bug by *Allotropa utilis*. These are among the most successful biological control projects in Canada. In each case only one species was introduced, not because this procedure was thought to have any special virtues, but because additional species were not known or available.

Now let us look at the other side of the coin. Pests that have long been established in a region usually attract a complex association of many biotic agents of regulation; their relations with their environment are complex, involving the interactions of many organisms. Multiple- and super-parasitism are common, and competition between agents must be intense, so intense that few, if any of them, can operate at full efficiency; indeed agents may often be limited in abundance by interspecific competition, and the food supply, i.e., the host, may not be fully exploited and may thus be able to increase in abundance. The spruce budworm, the black-headed budworm, and the hemlock looper are natives of North America that have evolved simultaneously with their environments. They are major pests of Canadian forests. But the spruce budworm, for instance, has over 35 known parasites as well as a host of predators. Varley (208) suggested that such insects may be pests because of, rather than in spite of, the large complex of organisms attacking them.

The complex of biotic agents that attack a long-established and well-adapted host insect, such as the spruce budworm, are part of a community that is in good natural balance with its environment. Other parts of this community are the host insect itself (in our view, a pest), and the host plant (in

our view, an economic product). There is no doubt that the spruce budworm is an integral part of and in good balance with the northern coniferous forest; and infestations are probably normal occurrences linked with the disposal of mature stands to make way for new growth (50, 150). It destroys trees, so to us it is a pest. But from an ecological point of view it is destroying only individuals of certain tree species which, were they not destroyed, would soon dominate all forest sites. In the absence of such agents all natural plant communities would progress to the true climax type and many species, both plant and animal, would be eliminated.

When an exotic species invades a new environment it upsets the natural economy as well as man's. The natural economy will eventually adjust to accommodate the newcomer, but in doing so will not necessarily produce a situation acceptable to us. We would like to reserve certain organisms, such as trees, for our own use, an arrangement which natural regulating forces are unlikely to support. In adjusting the situation to our needs, therefore, we cannot count on natural forces to promote our cause. The devious and subtle devices that bring balance to complex communities serve only to inhibit the achievement of our objectives. We hope and expect the pest species will become scarce. Thus two or more species of biotic agents exploiting this scarce commodity are almost certain to enter into competition. The more species of agents that attack the pest the sooner competition will start and the more severe will be its effects. We should therefore be wary of introducing large numbers of different organisms to attack a single host. A single efficient organism introduced without competitors would appear likely to serve our purposes best. Assuming that it possessed an adequate reproductive potential, and adequate searching efficiency, was synchronized with its intended host, and was suited to the physical environment of the new area, such an organism could multiply without restraint to the limit of its food supply. In such a situation population oscillations will almost inevitably be set up, but if the biotic agent possesses characteristics as defined, the peaks of pest numbers should not be excessive.

The proposition that two or more biotic agents attacking a single host species may interfere with each other to the advantage of host survival requires amplification. Most authors (15, 53, 197, 198, 200) refer to Smith's (170) statement that such interference is unimportant. Smith analyzed the possibilities of competition between two species of parasites attacking a single host and reasoned that only when the species that is the "inferior" of the two in the external environment is the survivor of all cases of multiparasitism is there any chance that host survival will be increased. After examining this situation he concluded that even though the "inferior" species may interfere with the "superior" species, each of the two species would occupy somewhat different niches and thus each would parasitize a portion of the host population unavailable to the other. Together the two species would therefore produce a greater accumulative mortality of the host than either species could alone.

Smith's reasoning followed these lines: a species introduced into a new environment will increase until it occupies all the niches available to it; it then "has become stabilized in its environment and the population has become constant within the limits of normal fluctuation"; during the period of population

expansion and up to the point of stabilization the "prolificacy" (Smith's term) of a parasite has an important bearing on the speed at which the population reaches stability, but once stabilization is achieved, "prolificacy" is of minor importance; from this point on the parasite is required only "to replace parental stock" and "there is no increase"; since virtually every species of animal can produce many times the parental stock each generation, all species can withstand exceedingly high mortality in any generation without a decline of numbers in subsequent generations; therefore, unless mortality due to interference by competing species is severe it will not seriously reduce the numbers of a parasite.

The concept that an environment contains a finite number of available niches that are occupied by a certain species of animal is unhelpful. As many factors, including weather, climate, biotic enemies, and the inherent characteristics of the species itself, are all known to influence population size, it is apparent that according to this concept the niche occupied by a species must be a compound of all the permanent and temporary factors that impinge on the species. As many factors are thus pooled in the niche concept, any particular factor may be obscured, and generalities expressed in terms of niches may lead to confusion when any one factor is considered individually. Smith expressly exempts "prolificacy" of a parasite from any influence on the determination of population numbers; therefore, he eliminates "prolificacy" from his concept of the niche. By "prolificacy" Smith apparently means the capability of the adult to lay eggs. Most insect parasites cannot lay eggs unless they find a host in which to lay them. Therefore "prolificacy" must include the capability to find hosts. Hosts are a major element of the niche occupied by a parasite. A niche is unavailable unless it can be found. Therefore "prolificacy" is an important factor in the availability of niches, and thus, according to Smith's own concept of population regulation, an important factor in the determination of the size of parasite population.

When Smith stated that an introduced species will increase until it fills all the niches available to it, he was really claiming that its population will increase until a number of environmental factors acting together check the increase. Then, he claimed, the population will "become constant (within the limits of normal fluctuation)". This parenthetical clause is the saving grace of this statement. It is generally accepted now that these "normal" fluctuations are the dominant feature of host–parasite interactions (137, 145, 200). The object of introducing parasites is to reduce the number of hosts, i.e., to reduce a critical part of the niche occupied by the parasite. How a parasite can reduce the number of niches available to it and still maintain a constant population is difficult to see. The interaction between a parasite and its host is essentially dynamic and both populations must continually be moving either up or down. If a parasite population remains steady it cannot cause the host population to decline, and if it causes the host population to decline it cannot remain steady. Smith's proposition that a parasite population remains steady is therefore untenable.

Now, let us look at the case Smith examined. Parasite A is "superior" to parasite B outside of the host, but when both parasites occur in a single

host only B survives. It is a simple matter to construct a mathematical model in which A is given an advantage over B in one or more external features, but in which only B survives in cases of multiparasitism. Such models are of doubtful value as no realistic parameters are known. In particular we have no valid information regarding host-finding by parasites; thus we have no way of estimating the amount of multiple parasitism. The best we can do is to assume random searching by both parasites and on that basis calculate the probable number of hosts that will be discovered by both species. In such models, B inevitably replaces A shortly after the host population begins to decline, even though the rate of increase of B is less than that of the host, and thus B has no chance of controlling the host by itself. The reasons are simple: when total parasitism is low, multiple parasitism is also low, and mortality to A from this cause is insignificant; thus A increases until it parasitizes a large percentage of the host population; meanwhile B builds up a modest population; finally, owing mainly to the efforts of A, the host population begins to decline until its numbers are reduced to approximately the same as, or less than, the numbers of parasite B; at this point virtually all surviving hosts will be parasitized by B as well as A; thus virtually all emerging parasites must be B. The host will be temporarily reduced in numbers, but as A, the only parasite of the two capable of controlling the host, is almost eliminated, the host is left to increase again.

Such deterministic models are admittedly unrealistic. The results that they indicate would occur only if the conditions of the models were fulfilled in nature. In particular, the above situation would hold only where every host is equally available to both species of parasite. If a portion of the host population was available only to A, another portion to both A and B, a third to B only, and a fourth unavailable to either, then presumably both parasites and the host would survive. This is a far more likely situation, but one of so many possibilities that it is impossible to predict the course of events without a thorough knowledge of the characteristics of all the species. But it seems evident that, where an "inferior" species has the ability to eliminate and replace any substantial part of the population of a "superior" species, the efficiency of the "superior" species must suffer. It was apparent from the mathematical models, though the host was greatly reduced in each case, that the host population rose higher and took longer to reduce when two competing species were employed than when only one parasite species was used.

Moreover, there is always the possibility that parasites may compete for a commodity in the external environment. Smith emphasizes niche differences to discount this possibility. But species may occupy niches that differ in every respect but one and come into critical competition within this common element. Adult food may be one such area of competition. Many adult parasites must feed to complete the development of their ovaries. Nectar, one of the foods required, may be scarce at certain times or in certain environments, so that there may be severe competition for this limited but essential commodity (91, 110). If parasite B emerges from the pupa a few hours before A, the latter species may find the nectar supply greatly depleted and thus be seriously inhibited in the production of eggs. If A is a "good" parasite and B a "poor"

one, this competition could seriously inhibit the ability of the "good" parasite to regulate the host at a satisfactory level, and the "poor" parasite may be inherently incapable of doing so.

This discussion, of course, does not prove that a single parasite species is better than two species. But it does show some of the inadequacies of Smith's reasoning, and suggests that Smith's conclusions are open to doubt. For a good many years now Smith's opinion has been accepted as virtually authoritative and the subject has been given practically no attention.

An important feature of this discussion is the question of what constitutes a "good" or "superior" parasite. Smith judged the efficiency of a parasite by the number of niches it is capable of occupying. We judge it by the number of pest hosts it is capable of parasitizing. Which of these is more nearly correct depends entirely on the point of view with which the parasite is judged. From the ecological, evolutionary viewpoint Smith probably comes closer to the truth; his criterion of efficiency is more likely to produce overall stability and survival of the species, i.e., it is more likely to produce a "natural balance". But as previously pointed out we are not concerned with a "natural balance". We are concerned with a balance that we hope to impose to satisfy our requirements. For this purpose we require quite a different flora and fauna than that which nature provides. Thus we do not necessarily want the parasite that is best adapted to the natural environment; instead we want one that will best do a specific job for us, and that is to prevent certain species from increasing beyond a certain level of abundance. Therefore, a high ability to parasitize a specific host species is an important attribute of a good parasite *for biological control purposes*. The ability to successfully compete with other parasites is of course another important attribute, but this attribute is only important when competitors are present. If we have a choice between introducing a highly "prolific" and otherwise successful parasite that is a poor competitor, and a parasite of low "prolificacy" but a good competitor, we can take advantage of the high "prolificacy" by introducing the parasite possessing this attribute without also introducing the competitor.

But there are cases where multiple importations of parasites have produced excellent controls. In many of these cases the actual control was achieved by a single organism the success of which, at least theoretically, could have been predicted; the remaining species failed to survive in the new territory. In other cases several species may have become established but one assumed the dominant role in the control. In still others several species became established and apparently achieved and maintain control by a "division of labor", as it were. The spruce sawfly project is said to represent the latter type of control and as this is often quoted as a justification of the multiple introduction method, let us examine it more closely.

One of the advantages of multiple introductions is said to be that when a problem of great urgency arises something can be done quickly. This is only valid if the "something" is useful. The spruce sawfly problem was recognized in 1930. Fifteen years later, after a prodigious amount of work, the insect seemed to be satisfactorily controlled, largely through a completely fortuitous circumstance. If the first few of those 15 years had been spent in sound observation and experimentation, much of the work and expense might have been

avoided and the same happy conclusion reached at an earlier date. Twenty-seven parasite species were released against *D. hercyniae* but only five became established on this host. A virus disease, unintentionally introduced and established, caused the crash of the epizootic population. Two of the established parasites, but principally one, *Drino bohemica*, have prevented further increases in the host population. Practically all of the species that failed to become established were primarily parasites of sawflies other than *D. hercyniae* or its close European relative, *D. polytomum*. They were introduced because the European literature indicated that they commonly parasitized a wide range of sawflies and therefore could presumably attack *D. hercyniae*. Laboratory tests confirmed this assumption. As the identities of many of these parasites were thoroughly confused at the time their host records were acquired, errors could be expected. In addition, it is now apparent that many of the parasites established on *D. hercyniae* and other sawflies are more host-specific than was originally thought.

Drino bohemica was reported to be a polyphagous parasite of sawflies and some Lepidoptera (139). The material for releases was reared from *D. polytomum*. It proved to be conspicuously effective in keeping *D. hercyniae* populations down once they were depressed to a low density by the virus disease. But *D. bohemica* failed against *N. sertifer*, and has rarely been recovered from sawflies other than *D. hercyniae*, though it is well established in areas where other sawflies occur.

Exenterus confusus was successfully established on *D. hercyniae* and was a factor in the regulation of the pest at high population densities, but declined in importance as the pest population declined. It was recovered only once from another host, a single collection of *Neodiprion abietes* (Harr.) from northeastern New Brunswick.

Exenterus vellicatus was successfully established on *D. hercyniae* and is recovered in appreciable numbers at low population densities. It has never been recorded from other sawflies in Canada.

Exenterus tricolor is said to be a parasite of *D. polytomum* in Finland and of *N. sertifer* in central and northern Europe. Small numbers were released in Canada in 1940. No recoveries were made until 1948, when a few specimens were obtained from *D. hercyniae* near Fredericton. It has not since been taken in Canada. If established, it has no effect on *D. hercyniae* populations.

Exenterus amictorius seems truly polyphagous on sawflies in Canada. It is established at low levels of abundance on *N. sertifer*, *N. nannulus nannulus* Schedl, *N. lecontei* Fitch, *N. virginiana* Rohw., *N. swainei* Midd., and *D. hercyniae*.

Dahlbominus fuscipennis was released for 12 years in massive numbers—almost 900 million individuals against *D. hercyniae* and more than a million against *N. sertifer*. Up to 1943 it was recovered from *D. hercyniae* in large numbers. Around 1943 *D. hercyniae* populations declined precipitously and releases of *D. fuscipennis* were suspended. No recoveries of the parasite from *D. hercyniae* were made after 1945. This has been attributed to a failure of *D. fuscipennis* to thrive at low levels of the host. But this parasite commonly

survives in Canada on low densities of *N. swainei* and *N. sertifer*, so it is not low host density *per se* that caused its failure on *D. hercyniae*. It is perhaps significant that *D. fuscipennis*, though commonly reared from several European sawflies, is not a parasite of *D. hercyniae* or *D. polytomum* in its native home (138). This suggests that *D. hercyniae* is not a permanently satisfactory host and that this reason, not low host density, accounts for the failure of the parasite in Canada. If this is so, the initial apparent success on *D. hercyniae* may have been a product of the massive releases of this parasite, plus its remarkable rate of increase, and it may never have been truly established on *D. hercyniae*.

Thus in spite of the 27 parasite species collected, propagated, and released against *D. hercyniae*, only two contribute significantly to control; of these *D. bohemica* is by far the most effective. The virus disease persists but is ineffective at low host densities. In all, therefore, this project is not so very far from the single species concept.

In summing up this section we feel that the introduction of many species of biotic agents with the hope that at least one of them will possess the attributes required to control a pest is indicative of the immaturity of biological control. In ages past, medicine was practiced in this way. Ailing patients were dosed with concoctions compounded from a weird variety of elements which in the imaginations of physicians should have had some effect on the ailment treated. Since those days medicine has achieved a more rational basis; now specific remedies are applied for specific ailments, and the proportion of successful recoveries has increased significantly. This is not to say that medical science now knows everything about the physiology of diseases, or how each drug or antibiotic works. But by a process of rational selection and testing of remedies in controlled experiments a good deal was learned, and physicians are now able to prescribe with discrimination. In biological control we are but slightly in advance of medieval medicine. Surely in this age of enlightenment we do not have to pass through a hundred years of darkness before we develop a rational approach to our problems. By introducing one carefully selected agent at a time and making adequate observations of what follows we can learn much about the attributes and deficiencies of the selected agent, and make further selections with these characteristics in mind. At the end of each project we will have thus accumulated some knowledge that can be applied to future projects, and will have constructed some basis for a rational application of appropriate measures.

Introduction and Release of Selected Organisms

When organisms have been carefully selected for introduction, how should we proceed with their movement and release to provide the best chances of success? Many problems arise: at how many points shall we release them and how many individuals should be released at each; over how many seasons or host generations should we make liberations; at what time of the year should these be made, and at what time of day; what sort of weather is best, or is there any particular sort of weather we should try to avoid; should we try to propagate the organisms received to increase their numbers? Because past releases were not made on a sound experimental basis, experience provides us

with few answers. Some remarks can be made on some of these problems, but they are based more on common sense than on experience.

The number of individuals to be released depends largely on the number available, but there should be some common sense upper limit. In the Canadian record there are two cases when this upper limit was exceeded: almost 900 million *Dahlbominus fuscipennis* were released against *Diprion hercyniae* in eastern Canada over a period of 12 years and almost 4 million *Bracon brevicornis* against *Ostrinia nubilalis* over a period of 11 years. Both of these parasites were intensively propagated by the greater part of the labor force of the Belleville laboratory throughout the periods of releases. During these periods, field research was almost entirely neglected. If establishment of these parasites on their respective hosts had been possible, fractional releases for about half the time would have sufficed. It must have been apparent early that economic control of the corn borer would not be achieved with *B. brevicornis*, since recoveries of this parasite, if not nil, were confined to no more than one or two isolated instances. *D. fuscipennis* was recovered from the spruce sawfly in large numbers shortly after the first releases. If it was going to become established and multiply it would have done so on its own initiative from that point, and only small additional releases to accelerate its spread would have been required.

Parasite releases, however, if not immediately successful, should be extended over a number of years. One failure does not necessarily mean that the parasite in question is unsuitable; the time, place, or method of release may have been wrong, or the insect may have been inhibited at a critical stage by unfavorable weather. No definite number of years or generations can be stated; each case should be judged on its own merits. If an organism persistently fails to become established, every effort should be made to discover the causes of the failure. If the cause is beyond our influence, the attempt should be abandoned.

The number of points at which parasites are released and the number released at each point are mutually interdependent and both depend on the number of individuals available. Obviously, the more release points the fewer the number of individuals released at each. Every attempt should be made to select sufficient release points to cover any possible range of host variation as well as variations of the environment. Experience can tell us very little about the number of individuals required to ensure success of a colony: *B. brevicornis* failed to become established from many colonies each of many thousands of individuals while *Opius ilicis* succeeded from a single release of only 10 individuals. As a rule of thumb, however, it is probably better to release larger numbers of individuals at a few points than to scatter minute colonies over a wide range. Populations must be sufficiently dense to permit the sexes to find each other. Of course, parthenogenetic parasites can be released in smaller colonies than those dependent on sexual reproduction.

The time of year for releases depends on the life-histories of the host and parasite. Synchronization of the life-histories of the two is essential, i.e., the appropriate stage of the parasite must be released when the appropriate stage of the host is most abundant in the field. This elementary principle has not always been followed. Adult *Blastothrix sericea* were released against the European fruit lecanium in mid-July, although the appropriate stage of the host is

not available until late autumn. However, the parasite adults apparently survived the intervening period and successfully parasitized hosts as they became available, but this fortunate circumstance does not lessen the error. Such maladjustment of synchronization may have been responsible for the failure of many parasite species.

There is practically no information regarding the best time of day or kind of weather for release. Ideally parasites should be able to commence activity as quickly as possible. Any condition of light, temperature, humidity, atmospheric electricity, or other meteorological factor that delays or prevents this places the infant colony in jeopardy.

Finally, there is the problem of whether organisms should be released as received or propagated to increase their numbers. Factors to be considered are the number of organisms received, the number required, the ease of propagation, and the labor and facilities available. In general, we are opposed to extended laboratory propagation as it must enforce a heavy selective pressure on the population, and it is doubtful whether the organisms produced are the same as those originally received. In some cases this might be an advantage; in others it would not. Information on this is almost entirely lacking and accordingly we must regard the possibility as a danger. At the same time, we should point out that in California, where laboratory propagation is frequently used, there has been little indication of harmful effects.

Common Misconceptions

After the initial, intemperate enthusiasm for biological control wore off, the method assumed a proper and logical place in applied entomology and performed many useful services. After World War II, however, the new and potent organic insecticides were introduced and biological control became neglected. This story has been related many times. Growing concern at the actual and potential dangers of these insecticides is now stimulating a reawakening of interest in biological control under the generally held impression that this method has fewer inherent dangers and is unlikely to upset the "natural balance" of populations. There is a widespread misconception that "biological control" and "natural control" are synonymous, and that anything that is natural is inherently, and perhaps morally, better than anything unnatural or artificial.

A control program that employs either toxic chemicals or biological agents constitutes a man-made manipulation of the regulatory complex of the pest species. The objective is to add mortality to the existing system. This manipulation can hardly be considered natural and thus the biological method of control is really no more "natural" than is the chemical. The fact that "natural" (i.e., living) rather than "unnatural" (i.e., non-living) agents are used to reduce populations is undoubtedly responsible for the view that biological control is natural, but this view is hardly justifiable. Insect problems rarely arise in natural situations, but rather in unnatural situations that have been created by injudicious population manipulation or interference by man.

One of the oft-quoted advantages of biological control is its permanence. A successful agent of biological control is always present to suppress any tendency of the pest species to increase again to become a serious economic problem.

It is precisely this characteristic that constitutes the gravest danger of biological control attempts. Mistakes that are made are also self-perpetuating; an organism, once successfully imported and established, is not easily eliminated if it proves deleterious to the solution of the problem. We do not mean by this, of course, that parasites and predators may "escape" and in their new homes destroy something that we wish to conserve, but rather that we are ignorant of the impact of these introduced species on their new environments and of the ways in which they may affect one another to the detriment of the control that we are trying to achieve. We will even say that when a problem offers opportunities for either chemical or biological control, the chemical method is preferable until such time as we have some idea of what the consequences of our actions may be. Before new introductions are undertaken, we feel that the problem should be carefully examined and one should proceed with caution. We are not, of course, condemning biological control, nor do we doubt its advantages and virtues; it is a powerful weapon if properly used. We do, however, condemn the way that it has often been injudiciously used in the past and we advocate its intelligent use in the future.

Another common misconception is that the mortality caused by a newly introduced agent of biological control is indicative of its importance. This has been rebutted a number of times but it still persists. Many mortality factors act on any insect population and eliminate a large portion of the individuals before they mature and reproduce. These are the factors that regulate all populations. The addition of a new factor may create a regulatory complex that is effective in regulating the population at a lower density and the importance of this factor could not be disputed. Its magnitude will reflect, however, conditions prior to its introduction and the level at which the other factors regulated the population in its absence. The new factor may be entirely additive, in which case it might well appear insignificant, or it might largely replace some existing factors. In the latter event, the new factor might appear very large but the *effective* part of the new mortality might still be small. For this reason, percentage parasitism as an index of the importance of an agent of biological control may be very misleading.

Summation and Suggestions

In our review of records and literature for this paper we were gratified at the percentage of documented cases that were successful. However, this degree of success is no cause for complacency; our failures still outnumber our successes. In his recent book, Elton (61) showed that it is a common characteristic of introduced species briefly to increase enormously and then to subside to a fairly static, lower level. Most of the subjects for biological control in Canada were exotic and this cycle may well have happened in many cases. If so, the decline was probably largely accomplished by an adaptation of native parasites and predators to the intruder. We must, therefore, be wary of taking credit for controlling insects biologically unless there is fairly strong evidence to support the claim. It is probably impossible to *prove* that any specific agent or group of agents produced control in the past and it is, therefore, impossible to prove that control was a result of any deliberate act on

our part. But in many cases it is possible to show beyond reasonable doubt, and in the absence of evidence to the contrary, that control resulted from some specific and deliberate act.

But what was most disturbing were the 29 attempts at biological control that were treated so casually that no adequate records were kept, and neither success nor failure can be shown. Some pests against which action was taken subsequently declined in numbers, and some of them even ceased to be pests, but we simply do not know whether this decline was related to the measures employed.

Nearly every aspect of entomology that deserves recognition as an entity has progressed from an observational to an experimental, analytical level. Biological control has been slow to do this. As Smallman (165) pointed out, research on principles is slow to accumulate knowledge and is an act of faith when undertaken in the hope that it will lead directly and rationally to the solution of practical problems. When direct experimentation leads to practical solutions, the immediacy between them tends to obscure the fact that both rest on a foundation of systematized knowledge and principles. The principal role of fundamental, in relation to applied, research is to provide rational explanations for empirical observations and thus to build them into the permanent structure of knowledge. This then provides fresh starting points for launching further empirical probes. Smallman concluded that this interplay between the search for principles and the solution of practical problems is necessary for the advancement of research. Our trouble in biological control is that we have not been consolidating the information received from our empirical probes. Consequently we have no rapidly growing foundation of knowledge and our more recent probes are penetrating the unknown little further than probes launched 20 to 30 years ago.

Applied control, biological as well as otherwise, should be the culmination of a broadly based, intensive program of fundamental research. Observational work has its place, of course, in that it suggests and reveals matters which should be intensively studied by experimentation and analysis. Prediction, an ultimate goal in biological control work, however, can be based only on experimental data: no amount of observational data is conclusive.

Unfortunately we do not in general have the basic knowledge to make sound predictions. Recommendations and predictions made in the past, even when based on thorough studies, usually fell wide of the mark. We need research: fundamental, long-term research on the principles of population regulation and interactions. In biological control this statement is rapidly becoming a cliché. Workers have been saying it for years, but most of them stop at saying it. We do not imply that no research has been done or is being done. There has always been a core of earnest and intelligent researchers in the field of population regulation and biological control, but the workers are pitifully few compared with the amount of work that needs doing. So far we have had only a glimpse at the inner works of population regulation. While the complexities of what we can see are as baffling as they are fascinating, it is rapidly becoming apparent that order does exist in population interactions and that it is amenable to resolution through organized observation and experimentation.

In the development of our personal research programs, we have evolved certain lines of thought that may be considered the basis of our present approach to biological control. Biological control workers have two tasks: they must seek the principles that underlie this method of control; and they must at the same time attempt to apply practical control measures. These two tasks impose a degree of dichotomy on our work: there is the practical, *ad hoc* work that involves the introduction and release of foreign, beneficial organisms; and there are the basic studies of a long-term nature that might be called fundamental ecology. Let us emphasize that we do not consider this dichotomy a bad thing. We feel that the two approaches should be complementary, and we should encourage the "interplay between the search for principles and the solution of practical problems" (165).

We have previously said that we do not regard *ad hoc* applied work as sound in view of the dangers inherent in its practice. We are not retreating from that stand when we say that such work is necessary at present. We have immediate problems that we must try to solve immediately. Practical controls cannot be put aside indefinitely until we have accumulated enough information to attack them on purely rational grounds. But we plead that *ad hoc* work be confined to essential situations, that it be applied with caution, and that, in so far as possible, it be designed to yield a maximum of usable information.

Let us not, however, be guilty of overemphasizing long-term, so-called "basic" research and thus arrive at another unbalanced state. There is a danger of the pendulum swinging too far from the empirical, applied approach. Long-term research must never become an end in itself, but must constantly be directed at the roots of practical problems. Our urgent need is for intensive information on certain limited subjects, and we should drive toward these objectives at the expense of immediately bringing up the whole front of knowledge. With a broad, but not necessarily complete, understanding of a few strategic subjects we should be able to direct our practical work more efficiently.

What, then, are the limited objectives we should strive for? In essence, applied biological control, and this is our ultimate purpose, hinges on being able to *select* species that will fulfill our needs, and to import, rear, and release these species with a maximum of success and a minimum of expense, time, and effort. Any process of selection can only be based on knowledge, and there are several fields of knowledge essential to the selection of natural enemies for our use. In brief, we must know much about the attributes that make an organism effective in limiting the abundance of others, how this effect is modified by undesirable characters, the mechanics of interactions, and, of course, the way in which the introduced species will fit into its new environment and its chances of survival there, and the attributes that determine this. We must study the modes of action of parasites, predators, and pathogens in much the same way that others at present study the modes of action of insecticides. At the same time, we should try to assess the general role that one can expect certain types of natural enemies to play and to determine the ways in which we may expect these to interact with their environments.

The conscious development of a dichotomous approach to biological control has a danger that we want to expose in conclusion. Workers on long-term

projects frequently develop an attitude that leads them more and more to appreciate their work as an end in itself. They may lose sight of their aims and direction and cease to think of their work as it contributes to the subject being considered. This will tend to drive this sort of project into basic ecology or pure biology. Similarly, workers on shorter-term, applied projects get further and further from science and are led to studies of *ad hoc*, or empirical, control. If this happens in biological control, its practitioners would come to belong primarily to the fringes of other disciplines, and this we feel is wrong and should be resisted. Biological control is sufficiently specialized to warrant consideration as a science in its own right, one that may draw heavily on other disciplines but no more. Its parts should be directed inwards to its own unique problems, not outwards to the problems of other subjects. In this way we may integrate our efforts, keeping our eyes firmly on both practicality and the urgent necessity for more-fundamental work that will place our practical work on an intelligent level. Perhaps with this balanced, focused approach we may reach the happy state where, in Charles Elton's words "Mass destruction [by chemicals] and the casual releasing of predators and parasites may some day be looked back upon as we do upon the mistakes of the industrial age, the excesses of colonial exploitation or the indiscriminate felling of climax forests" (61).

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Appendix

Taxonomic Position of Insects Discussed in This Paper

Adelges nusslini (Börner) Hom.: Adel.
Adelges piceae (Ratz.) Hom.: Adel.
Agathis pumilis (Ratz.) Hym.: Brac.
Agrothereutes abbreviator (Fab.) Hym.: Ichn.
Aleochara bilineata Gyll. Col.: Stap.
Aleochara bipustulata (L.) Col.: Stap.
Allotropa utilis Mues. Hym.: Platy.
Ancylis comptana (Fröh.) Lep.: Olet.
Apanteles lacteicolor Vier. Hym.: Brac.
Apanteles solitarius (Ratz.) Hym.: Brac.
Aphaereta pallipes (Say) Hym.: Brac.
Aphelinus mali (Hald.) Hym.: Eulo.
Aphytis maculicornis (Masi) Hym.: Eulo.
Apistephialtes caudata (Ratz.) Hym.: Ichn.
Ascogaster quadridentatus (Wesm.) Hym.: Brac.

Bessa harveyi (Tnsd.) Dip.: Tach.
Bigonicheta spinipennis (Meigen) Dip.: Tach.
Blastothrix sericea Dalm. Hym.: Enc.
Bracon brevicornis Wesm. Hym.: Brac.
Bruchus pisorum (L.) Col.: Bruc.

Calosoma sycophanta (L.) Col.: Cara.
Carpocapsa pomonella (L.) Lep.: Olet.
Cephus cinctus Nort. Hym.: Ceph.
Cephus pygmaeus (L.) Hym.: Ceph.
Choristoneura fumiferana (Clem.) Lep.: Tort.
Choristoneura murinana (Hbn.) Lep.: Tort.
Chrysocharis pubicornis (Zett.) Hym.: Eulo.
Chrysoplatycerus splendens (How.) Hym.: Enc.
Coleophora laricella (Hbn.) Lep.: Cole.
Collyria calcitrator (Grav.) Hym.: Ichn.
Compsilura concinnata Meigen Dip.: Tach.
Cremifania nigrocellulata Cz. Dip.: Cham.
Cryptus sexmaculatus Grav. Hym.: Brac.
Cyrtogaster vulgaris Wlkr. Hym.: Pter.

Dacnusa gracilis (Nees) Hym.: Brac.
Dahlbominus fuscipennis (Zett.) Hym.: Eulo.
Dendroctonus piceaperda Hopk. Col.: Scol.
Dicladocerus sp. Hym.: Eulo.
Diprion hercyniae (Htg.) Hym.: Dipr.
Diprion polytomum (Htg.) Hym.: Dipr.
Diprion similis Htg. Hym.: Dipr.
Drino bohemica Mesn. Dipt.: Tach.

Elodia tragica (Meigen) Dipt.: Tach.
Encarsia formosa Gahan Hym.: Eulo.
Epiblema strenuana (Wlk.) Lep.: Olet.
Epilampsis gemma (Wlkr.) Hym.: Eulo.
Epilampsis laricinellae (Ratz.) Hym.: Eulo.
Eriosoma lanigerum (Hausm.) Hom.: Aph.
Eulecanium coryli (L.) Hom.: Cocc.
Eupteromalus nidulans (Thoms.) Hym.: Pter.
Evagora milleri (Busck) Lep.: Gel.
Evagora starki Free. Lep.: Gel.
Exenterus amictorius (Panz.) Hym.: Ichn.
Exenterus confusus Kerr. Hym.: Ichn.
Exenterus tricolor Rom. Hym.: Ichn.
Exenterus vellicatus Cush. Hym.: Ichn.

Forficula auricularia L. Derm.: Forf.

Glypta haesitator Grav. Hym.: Ichn.
Glypta rufiscutellaris Cress. Hym.: Ichn.
Grapholitha molesta (Busck) Lep.: Olet.

Hemisarcoptes malus (Sheiner) Acarina
Heterospilus cephi Rohw. Hym.: Brac.
Horogenes nanus (Grav.) Hym.: Ichn.
Horogenes punctorius (Rom.) Hym.: Ichn.
Hylemya brassicae (Bouché) Dip.: Musc.
Hylemya cilicrura (Rond.) Dip.: Musc.
Hylemya floralis (Fall.) Dip.: Musc.
Hylemya planipalpis (Stein) Dip.: Musc.
Hyperica postica (Gyll.) Col.: Curc.

Laricobius erichsonii Rosenh. Col.: Dero.
Laspeyresia nigricana (Steph.) Lep.: Olet.
Laspeyresia youngana (Kearf.) Lep.: Olet.
Lepidosaphes ulmi (L.) Hom.: Cocc.
Leptomastidea abnormis (Grlt.) Hym.: Enc.
Leptomastix dactylopii How. Hym.: Enc.
Leucopis obscura Hal. Dip.: Cham.
Lipoleucopis praecox de Meij. Dip.: Cham.
Loxotropa tritoma (Thoms.) Hym.: Diap.

Macrocentrus ancylivorus Rohw. Hym.: Brac.
Mantis religiosa L. Orth.: Mant.
Melanichneuman mucronatus (Prov.) Hym.: Ichn.
Mesoleius tenthredinis Mor. Hym.: Ichn.
Meteorus versicolor Wesm. Hym.: Brac.
Microctonus aethiops (Nees) Hym.: Brac.

Neodiprion abietis (Harr.) Hym.: Dipr.
Neodiprion lecontei Fitch Hym.: Dipr.
Neodiprion nannulus nannulus Schedl Hym.: Dipr.
Neodiprion sertifer (Geoff.) Hym.: Dipr.
Neodiprion swainei Midd. Hym.: Dipr.
Neodiprion virginiana Rohw. Hym.: Dipr.
Nygmia phaeorrhoea (Donovan) Lep.: Lym.

Operophtera brumata (L.) Lep.: Geom.
Opius ilicis Nixon Hym.: Brac.
Ostrinia nubilalis (Hbn.) Lep.: Pyr.

Parlatoria oleae (Colvee.) Hom.: Cocc.
Pediobius beneficus (Gahan) Hym.: Eulo.
Perilampus sp. Hym.: Peri.
Perilitus rutilis Nees Hym.: Brac.
Phenacoccus aceris (Sign.) Hom.: Cocc.
Phygadeuon sp. Hym.: Ichn.
Phytomyza ilicis Curt. Dipt.: Agro.
Pimpla turionellae (L.) Hym.: Ichn.
Pineus pini (L.) Hom.: Adel.
Pineus strobi (Htg.) Hom.: Adel.
Pristiphora erichsonii (Htg.) Hym.: Tent.
Pristomerus vulnerator (Panz.) Hym.: Ichn.
Pseudococcus citri (Risso) Hom.: Cocc.
Pseudococcus maritimus (Ehrh.) Hom.: Cocc.
Psila rosae (Fab.) Dipt.: Psil.
Pullus impexus (Muls.) Col.: Coccin.
Pygostolus falcatus Nees Hym.: Brac.

Rhizophagus sp. Col.: Nit.
Rhopalicus tutela (Wlkr.) Hym.: Pter.
Rhyacionia buoliana (Schiff.) Lep.: Olet.
Rodolia cardinalis (Muls.) Col.: Coccin.

Sarcophaga caridei Brethes Dip.: Sarc.
Sitodiplosis mosellana (Geh.) Dip.: Chir.
Sitona cylindricollis Fahr. Col.: Curc.
Sphegigaster flavicornis (Wlkr.) Hym.: Pter.
Stilpnotia salicis (L.) Lep.: Lym.

Sympiesis viridula (Thoms.) Hym.: Eulo.

Trialeurodes vaporariorum (Westw.) Hom.: Aleu.
Triaspis thoracica (Curt.) Hym.: Brac.
Trichogramma minutum (Riley) Hym.: Tric.
Tritneptis klugii (Ratz.) Hym.: Pter.
Trybliographa rapae (Westw.) Hym.: Cyn.

Zarhopalus corvinus (Grlt.) Hym.: Enc.

Key to Abbreviations of Family Names

Adel-gidae
Agro-myzidae
Aleu-rodidae
Aph-ididae

Brac-onidae
Bruc-hidae

Cara-bidae
Ceph-idae
Cham-aemyiidae
Chir-onomidae
Cocc-idae
Coccin-ellidae
Cole-ophoridae
Curc-ulionidae
Cyn-ipidae

Dero-dontidae
Diap-riidae
Dipr-ionidae

Enc-yrtidae
Eulo-phidae

Forf-iculidae

Gel-echidae
Geom-etridae

Ichn-eumonidae

Lym-antridae

Mant-idae
Musc-idae

Nit-idulidae

Olet-hreutidae

Peri-lampidae
Platy-gasteridae
Psil-idae
Pter-omalidae
Pyr-austidae

Sarc-ophagidae
Scol-ytidae
Stap-hylinidae

Tach-inidae
Tent-hredinidae
Tort-ricidae
Tric-hogrammatidae

FURTHER OBSERVATIONS ON AMOEBOID HAEMOCYTES IN BLABERUS GIGANTEUS (L.) (ORTHOPTERA: BLATTIDAE)[1]

J. W. Arnold

Abstract

The movements of amoeboid haemocytes in vivo in wing veins of *B. giganteus* were studied with the aid of time-lapse cinephotomicrography and projection analysis. They are described here in detail and discussed in relation to haemocyte form and function. Haemocyte motion included non-migratory as well as migratory aspects. Non-migratory motion comprised the slow to turbulent cytoplasmic motion and intermittent probing movements of stationary cells. Active migration occurred in the more or less typical amoeboid fashion and also in the more peculiar contractile manner which involved the projection of hyaline, tactile pseudopodia of variable form and often resulted in extreme and relatively rapid elongation of the cell body. Haemocytes were thus able to move on flat surfaces, to extend themselves across spaces, and to force their way into narrow tissue interstices. These activities demonstrate the versatility of the cells and provide a means of accounting for certain of their functions.

Introduction

Independent motion of insect haemocytes in vivo was previously demonstrated by the writer with the blood cells of the giant cockroach *Blaberus giganteus* (L.). Since the publication of the original observations (3) the behavior of the haemocytes in the wing veins of this insect has been studied further with the aid of time-lapse cinephotomicrography and projection analysis. This technique has helped to clarify some aspects that were not evident from direct observation and which contribute to an understanding of certain haemocyte activities.

Two principal methods of haemocyte migration, "typical amoeboid motion" and "atypical amoeboid motion", were distinguished in the original description (3). They were considered to be related activities that warranted separate descriptions, partly for the sake of clarity and partly to mark separate functional implications. This separation is now seen to be an oversimplification of the activities of the cells. Haemocyte motion, as a special form of amoeboid motion, includes the basic features of the latter along with some peculiarities that are not amoeba-like in the generally accepted sense. The motion is described here in detail and discussed with regard to haemocyte functions. Its relation to amoeboid motion per se and to the movements of the presumably homologous "amoebocytes" of other invertebrates and the leucocytes of vertebrates is considered briefly.

The literature on amoeboid motion of invertebrate blood cells is relatively brief and mainly incidental to general studies on the form and functions of the cells. Much of the early literature has been reviewed by Fauré-Fremiet (9) and by Haughton (13), among others, and includes the work of such major contributors as Loeb (19), Tait (24), and Tait and Gunn (25). More recently some pertinent references to amoebocyte motion were included in

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studies by Barer and Dick (4), George and Ferguson (10), Liebman (18), and Millara (21). In general the investigations suggest that the amoebocytes are seldom comparable in activity with free-living amoebae. Although real comparisons have not been made, the descriptions indicate that the cells are comparatively active in echinoderms, annelids, and molluscs, and feeble among the arthropods. It should be pointed out, however, that the investigations depended largely on in vitro techniques that have been patently unsuccessful for the study of insect tissues (see ref. 5) and are probably not entirely suitable for other invertebrates. Furthermore, the suggestion as it pertains to arthropod blood cells is not supported by the writer's observations on the haemocytes of *B. giganteus* in vivo (3) or by the studies of two early workers who used somewhat similar techniques with other insects. Moseley (22) remarked on the active amoeboid movements of haemocytes in the wing veins of *Blatta orientalis* L., and Dewitz (8), working mainly with *Tenebrio molitor* L., described extreme haemocyte activity in adult wings and in fresh in vitro preparations of pupal wings. Dewitz also noted that haemocytes were only feebly active when removed entirely from the insect body.

The literature on amoeboid motion per se is of course extensive, and descriptions and experiments, and new or modified theories, are continually appearing. Reviews on the subject have been published by several authors including De Bruyn (6) and Seifriz (23). For the most part the theories attempt to explain the mechanism of movements that involve protoplasmic streaming and the formation of blunt pseudopodia. Although the haemocytes of *B. giganteus* do move in such a manner on occasion, their advance just as often involves an obviously greater degree of contractility and the pseudopodia, like those of the amoebocytes of other invertebrates, are typically filiform or at least terminate acutely. From this standpoint the theories might be considered inapplicable to haemocyte motion. On the other hand the haemocytes might serve as a further example to support the contractility theories of amoeboid motion which are regaining general acceptance and for which considerable experimental and biochemical data have been obtained (e.g., refs. 15 and 16). Furthermore, although the fundamental mechanism for amoeboid motion may be common to all organisms, it is expressed in different ways even among the amoebae. Dellinger (7) described rather extreme differences in the manner of locomotion between species of *Amoeba* and *Difflugia* and noted subtle distinctions among species of *Amoeba*. Similarly Griffin and Allen (12) divided amoebae into polypodial, monopodial, and anteriorly flattened species on the basis of cell form and manner of attachment to the substratum during locomotion.

Materials and Methods

Adults of *B. giganteus* were used exclusively. Rearing and observation methods were the same as described previously (2, 3) with the addition of time-lapse cinephotomicrography and projection analysis. The cinephotomicrographs were prepared in cooperation with the bio-Graphic Unit, Research Branch, Canada Department of Agriculture, and with special assistance from cine-photographer Charles E. Beddoe. The camera was a 16-mm Cine-Kodak Special II mounted on the microscope. It was operated at one or at

two frames per second during the filming but these sequences are not maintained in the series selected here for illustrations. Bright-field illumination was used in conjunction with a Wratten B filter No. 58 (green). The original films were studied with a Kodak Analyst projector that provided variable speed and single-frame selection. The technique, which included projection onto graph paper and tracing cell outlines frame by frame, proved to be invaluable for detailed study of the cell movements and especially for following the actions of pseudopodia and cytoplasmic granules. Some of the illustrations are from series of still photomicrographs taken with electronic flash illumination as described previously (3). They were superior in detail and contrast to individual movie frames for the purpose of publication.

Observations and Discussion

Although all of the haemocytes in *B. giganteus* are at least potentially amoeboid the most active ones and those referred to specifically here are distinctive cells that may be termed "plasmatocytes" after Yeager (29), or "phagocytes", "amoebocytes", or certain categories of "leucocytes" of a number of other authors. This confusion in terminology was discussed briefly by Wigglesworth in his recent review on insect blood cells (26). When the cells are in the so-called passive condition, as they occur typically in the circulating blood of young, healthy adults, they are turgid and resilient and maintain a comparatively stable disk-like or fusiform shape. They are then about 20 to 30 microns in greatest diameter and distinguished by a central nucleus that occupies about one-third of the total cell area and cytoplasm that is finely and uniformly granular. Conversely, in the active condition, as exemplified especially during amoeboid movement, they display an unstable and varying shape that presumably reflects reversible physicochemical changes in all or parts of the protoplasm, which result in solation to a varying degree. The nucleus then tends to be eccentric and the endoplasm is often concentrated so that areas of hyaline cytoplasm are evident, particularly at the cell extremities.

It has long been recognized that the invertebrate amoebocyte can exist in either a passive or an active condition and that these conditions are reversible. This ability, as pointed out by Yeager (28) and others and as observed here with *B. giganteus*, is as characteristic of insect haemocytes as it is of the amoebocytes of the marine invertebrates in which it was first described. It is undoubtedly of fundamental importance in amoeboid movement as well as other activities of the haemocytes. Nevertheless, despite the apparent distinctions between passive and active cells, the two conditions are relative and not always easily identified. Passive cells displayed some degree of protoplasmic motion (Fig. 48) and active cells were often passive in form, at least intermittently. Furthermore, haemocytes in active form occurred not only during amoeboid migration but also freely suspended in the circulating blood, where they became more numerous as the insect grew older (2). From general observations this increase in haemocyte activity in association with age is considered to be due partly to the more frequent contact of the haemocytes with other tissues as a result of the general decline in the rate of blood circulation and partly to the presence in the blood of increasing amounts of some

factor, possibly a product of tissue degeneration, which stimulates amoeboid or other activity. The stimulating effect of contact with other tissues was evident simply from the tendency of the haemocytes to move independently when they settled on the walls of blind or partly occluded wing veins where the rate of circulation was obviously retarded. This is an extension of Tait's suggestion (24) that contact with a foreign body caused protrusion of pseudopodia. The presence of a stimulating humoral factor was suggested by two observations. In young adults, haemocytes in contact with a vein wall were slow to change, were generally less active than in old specimens, and seldom moved in veins where the blood current was swift. In old adults, on the other hand, a fairly large proportion of the circulating haemocytes were active in form and those that settled on the vein walls were often observed to move against a strong current that eventually carried them away. In moribund adults where wing circulation was much retarded almost all of the haemocytes were active to some degree. The situation seems comparable, on a larger scale, with the localized stimulation of diapedesis in vertebrate leucocytes by the polypeptide leucotaxine that results from protein breakdown at sites of acute inflammation (20).

As mentioned above, some protoplasmic motion occurred in passive cells. The motion was slow, apparently confined to small areas within the cell at a given time, and sometimes evident only from slight changes in cell form or in some rearrangement of the endoplasm during periods of several hours. This was particularly true for cells that were lodged in veins where the blood flow was swift. In other cases, where the cell was mainly stationary but could more correctly be termed active, slow protoplasmic movements could be observed directly. In its mildest form the motion consisted of a slow and apparently haphazard jostling of endoplasmic granules within small areas of the cell. In more active form the granules circled irregularly as individuals or as small chains that percolated in and out among a seeming meshwork of relatively stationary granules which themselves intermittently joined in the action or moved in an opposite direction. In still more active form the streaming contributed to a slow jostling or rolling motion of the entire cell within a small field and was sometimes accompanied by the intermittent projection and withdrawal of hyaline, filiform pseudopodia. This sort of rotation and a somewhat similar probing motion that persisted for long periods without active migration was observed frequently and especially in cells with a smaller than normal mass of granular endoplasm. To some extent this supports Tait's suggestion (24) that the power of amoeboid motion is in direct proportion to the number of granules in the cytoplasm. The suggestion was supported further by the observed prolonged quiescence of hyaline cells which occasionally moved very slowly over comparatively short distances and with little change in form.

Almost invariably the slow streaming of protoplasm and the rotating or probing actions of stationary haemocytes was succeeded by a brief period of protoplasmic turbulence immediately prior to migration. The turbulence appeared as a mild churning motion as though slow waves of contraction spread over the cell, and became more directed and continual as the cell began to move. Although turbulence is a common activity of protoplasm and serves

a number of vital cellular functions (23) it seemed to be concerned here especially with the organization of the protoplasm in some way to facilitate unidirectional advance. It also occurred invariably when a cell reversed its direction of movement. Like the vertebrate leucocyte (17) the haemocytes tended to maintain an anterior and a posterior end during migration. A complete reversal of direction involved considerable turbulence whether the cell actually turned around or, as occurred more commonly, simply halted and moved in the opposite direction. Presumably streaming and turbulence were prerequisite to migration but the immediate cause of migration was seldom evident. Occasionally it was initiated in a stationary cell by the pressure of an active cell squeezing past it in a narrow vein (Figs. 15 to 18). Again, simple contact with a vein wall seemed to initiate activity in some cells whereas others might remain in similar positions for long periods without moving. Stimulating humoral factors and/or the availability of energy for migration were probably involved. Glycogen and lipoids are known to occur intracellularly in some haemocytes (1, 26, 30) and are an obvious source of energy. The glycogen occurs particularly in plasmatocytes and is the more likely source since lipoids are stored mainly in haemocytes that are only feebly amoeboid.

The seemingly unorganized protoplasmic turbulence was succeeded by controlled cell movements of variable form that seemed to be related partly to extracellular factors, both physical and chemical, and partly to intracellular energy resources or to the distribution of contraction and relaxation factors within the cell. These suggestions derive from the frequent change from one "type" of motion to another (Figs. 1 to 10) and periodic cessation of movement either with an apparent relation to the cell's physical surroundings or where there was no obvious change in its environment. Measurements of the rate of migration were not entirely satisfactory owing to the tendency of the cells to stop and probe intermittently. However they usually averaged about five microns per minute with periodic speeds to seven microns per minute.

Movement of the haemocytes in monopodial amoeba-like fashion was described previously (3) and is illustrated more fully here (Figs. 11 to 14, and 30 to 32). Observations were not extended greatly, but it was noted that the cells moved in this way for appreciable periods mainly where they filled the vein in which they were travelling or where they were flattened against the substrate. Otherwise the motion was relatively short-lived and comprised an intermediate phase between longer periods of motion by other means. The two situations evoked somewhat different responses. Where it filled the vein (Figs. 11 to 14) the haemocyte was turgid and cylindrical with rounded ends. It moved very slowly with slow protoplasmic streaming and rather irregular contraction waves. Where the haemocyte was unconfined (Figs. 30 to 32) it appeared much less turgid and adhered closely to the substrate over its entire length. The anterior end tended to be broader than the posterior, the nucleus tended to be located near to it, and it was the site of most active granule movement. The posterior end often terminated as a lax, tail-like extension. In some respects haemocytes moving in this manner were more closely akin to migratory lymphocytes as described by Lewis (17) than to amoebae.

However they lacked the constriction ring that was involved in lymphocyte movement and they were not restricted to this manner of movement.

The most common form of haemocyte motion was that which was originally termed "atypical amoeboid motion" (3). Fundamentally it is a more active and controlled phase of the amoeba-like motion with some additional features that help to explain the ability of haemocytes to penetrate other tissues. The cells moved with typical, rather haphazard protoplasmic streaming and with obvious waves of contraction. The blunt anterior end of the cell body was almost continually prolonged as a hyaline projection of the ectoplasm that varied in size and shape from a short, pointed tip to a lamellar expansion or to a filiform pseudopodium up to about eight microns in length (Figs. 1 to 10). The continuous variation in size and shape of the pseudopodium indicated that some exchange occurred between it and the hyaloplasm of the cell, although granular material was mainly excluded. A few granules did continually move in and out of it in a shuttling motion, apparently as a result of the alternate waves of contraction and relaxation in the cell body, but they never moved in a stream. Periodically the pseudopodium disappeared as endoplasm entered the area but it usually extended again almost immediately at the same site. When it was lamellar, the hyaline pseudopodium tended to adhere firmly to the substrate. When it was filiform it was turgid and flexible and not adherent, attaching only when extended and then only at the tip, in the manner of the pseudopodia of carnivorous amoebae (12). In this condition the pseudopodium preceded the cell body (Figs. 19 to 26), following the contours of the substrate and moving from side to side as the cell advanced in a wobbling fashion and adhering intermittently. In this way it became inserted into narrow spaces or openings that the cell body might otherwise have passed by. It thereby directed the cell which had considerable impulsive force and pushed onwards into confined areas, often widening the gap as the nucleus entered. This action was assisted by an identical posterior pseudopodium (Figs. 19 to 26) that adhered at its tip to the substrate and provided a base from which the cell extended itself forward. It released its attachment and was sometimes reabsorbed when the cell body moved ahead sufficiently to stretch it. Where the passageway was very narrow and inflexible much of the protoplasm was forced ahead of the firmer nucleus which thereby came to lie close to the posterior end of the cell. This was only a temporary condition if further progress was unimpeded. However, if it was blocked, the cell simply withdrew with the original posterior end now leading. Occasionally haemocytes were observed to force cytoplasm ahead of the nucleus and through spaces only slightly wider than a granule of the cytoplasm, i.e., of the order of 1 micron (Figs. 44 to 47). In each case however the cell eventually withdrew from the site.

Haemocytes were observed to migrate in the above manner in a variety of situations within the wing, both where they were unconfined in large veins and also where the veins were narrow or partly occluded so that the cell's movements were restricted. It seems reasonable to suggest that they behave similarly throughout the body and that the mechanism is responsible for their penetration into the cellular interstices of other tissues. The same mechanism also enabled them to proceed rapidly in open areas in short

"jumps" and to bridge gaps, e.g., from one side of a vein to the other or to the surface of a centrally located trachea. In some instances the haemocyte moved along the substrate during the rapid advance (Figs. 33 to 36) and adhered to it at the "tail" until the anterior made firm contact at the new position. In other cases the tail adhered firmly to the substrate while the cell body moved freely in the lumen of the vein (Figs. 37 to 43) as a wave of contraction spread from the posterior to the anterior end. This caused it to extend to about twice its normal length. When the free anterior end contacted a substrate it adhered to it. The posterior attachment then released as the cell relaxed and was drawn to its new position. Here the anterior pseudopodium was directly involved in the cell's progress and appeared to be modified so that several short, hyaline, filiform extensions emerged around the tip and presumably were involved in adherence to the substrate. Such behavior was observed mainly where the cell emerged from a narrow vein into an open one where the circulation was poor. Then the cells performed only two or three successive jumps before reverting to the more normal methods of progression or becoming stationary. Where the cell emerged into a vein where the circulation was strong (Figs. 27 to 29) it tended to release its attachments, become rounded in form and float away in the blood current. There was no obvious cause for the increased activity during rapid advance and it was observed in cells that were in company with others that moved normally. Possibly the exceptional extension resulted simply because the cell body had become free from the substrate so that the entire force of the contraction acted on the cell itself rather than against a solid surface. It was observed only in old adults where most of the haemocytes were active and this again suggests that a humoral stimulus was acting on cells that were in a physiological condition to respond very actively. In either case the action seemed to conform to the statements of Hoffmann-Berling (15) regarding cell movements in that the more active phase, that of extension, was the result of contraction. The slower phase that involved release of the original attachment and movement of the cell to its new position seemed to result mainly from relaxation.

In connection with these observations, haemocytes that were similar in size and form to those in the fully extended condition were frequently observed, apparently fixed in position and living, extending across veins where blood circulation had stopped. Their positions were later marked by structureless, fiber-like "ghosts" (Fig. 49) which sometimes formed a network in the veins of moribund insects and gradually disappeared after death. The condition was not unlike the meshwork of granular fibrils in the coagula of orthopteroid insects as described by Grégoire (11). It was also reminiscent of some aspects of connective tissue formation and of the conversion of haemocytes into capsule substance around metazoan parasites as reported by several authors (see ref. 26). Perhaps this is a mechanism that isolates areas where circulation has ceased.

It is noteworthy that the position of the main pseudopodia on the cell body tended to be restricted, and was apparently regulated to some extent by the organization of the protoplasm that occurred prior to migration. The pseudopodia almost always emerged from the ends of oval or fusiform cells, i.e., in line with the direction of motion, and, once formed, their positions

tended to be fixed. They re-emerged from the same sites after being absorbed into the cell body. Although it was not uncommon for two pseudopodia to be extended from the anterior end, especially where an obstacle was met (Figs. 30 to 32), this condition was temporary and flow was soon directed into one at the expense of the other. A similar situation also developed sometimes where the haemocyte was stationary and was projecting pseudopodia prior to active migration. It is also of interest that the haemocytes, with their ability to adhere to substrates on contact, normally had little or no tendency to adhere to each other or to become fixed to other tissues. Where two active haemocytes approached each other in a narrow vein, one or both of them reversed direction on contact or squeezed past if space permitted. Similarly, passive circulating haemocytes constantly jostled each other without adhering and clumps that formed at obstructions were usually simple piles of cells that tended to disperse gradually. On the other hand the tendency for haemocytes to adhere to each other and to other tissues increased with age (2) and was a constant feature of abnormal conditions, including the suspension of blood in vitro. It indicates rather drastic and mainly irreversible changes in the surface properties of the cells and doubtless serves as a defensive mechanism. Presumably sites of infection, of invasion by parasites, or of wounding would be isolated when active cells approached, either independently or in circulation, and agglutinated if conditions in the abnormal area caused surface changes to occur. This phase of protection would precede the fiber formation referred to previously.

The characteristics displayed by the haemocytes as they move independently in vivo, their sensitivity to their immediate environment, their ability to modify their form and their manner of locomotion, their capacity to extend tactile pseudopodia, to adhere to and release from the substrate, to force their way into narrow openings, to flatten or to extend themselves, or to assume a passive form and condition as circulating cells, provide direct evidence of their ability to perform certain of their assumed or accepted functions. Among the functions of insect blood cells Wigglesworth (26) considered five major activities, phagocytosis and immunity, protection from metazoan parasites, blood coagulation, connective tissue formation, and intermediary metabolism. From the present observations it is difficult to avoid the conclusion that the fundamental mechanism involved in haemocyte motion, the mechanism that affords them versatility and seems to distinguish them most obviously from cells of other tissues, is involved at some stage in each of these categories and is often an essential feature.

Despite the apparent distinctions between haemocytes and the cells of specialized tissues it is often difficult to distinguish one from the other. This is especially true where the haemocytes are in the active condition and closely associated with the other cells. There is good reason to believe that some of the primitive characteristics of haemocytes are retained by the more highly differentiated and less versatile cells and lead to this difficulty. It seems more than coincidental, for example, that the forms assumed by independently moving haemocytes, and particularly the extension of hyaline, tactile pseudopodia, are also assumed by cells of other insect tissues such as migrating chromatophores (14), epidermal cells that are attracting tracheoles to sites of oxy-

gen deficiency (27), and cells of various tissues cultured or dissociated in vitro (see ref. 5). Such similarities may account for and may perhaps lend some evidence to the repeated suggestion in the literature that the haemocytes are "embryonic" cells that are still subject to differentiation into specialized tissues. On the other hand it may simply indicate that the mechanism involved in haemocyte motion is a very fundamental one that is retained by cells of organized tissues and can be expressed under some circumstances.

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NOTE: Figures 1–49 and their captions follow.

Explanation of Figures

Figs. 1–10. A haemocyte moving independently through a vein complex where the circulation of haemolymph was negligible. Figures are of the same cell at intervals of approximately 5 minutes. Note the variation in cell form, position of the nucleus, the form of the pseudopodium, and the degree of adherence of the cell to the vein wall. Period of observation, 1 hour. Distance travelled by the haemocyte, 300 microns (approx.). Average speed, 5 microns per minute. Cell still moving actively at the end of the observation period. Photomicrographs, ×1000 (approx.).

Figs. 11–26. Selected frames and tracings from time-lapse motion picture of amoeboid haemocytes, ×500 (approx.).

Figs. 11–14. Haemocyte that fills the lumen of the vein advancing slowly without pseudopodia. Intervals between frames approximately 2 minutes but variable.

Figs. 15–18. Haemocyte that does not fill the lumen of the vein advancing with hyaline pseudopodia of variable form. Note the use of a filiform pseudopodium to insert the cell past a stationary haemocyte which also becomes active. Intervals between frames approximately 30 seconds but variable.

Figs. 19–26. Haemocyte advancing through an enlarged portion of a vein. Note the probing action of the anterior hyaline, filiform pseudopodium and the gradual absorption of an identical posterior "tail". Intervals between frames approximately 60 seconds but variable.

Figs. 27–32. Still photomicrographs and tracings of amoeboid haemocytes, ×1000 (approx.).

Figs. 27–29. Haemocyte emerging from a confining vein into one where haemolymph circulation was rapid. Intervals between figures approximately 60 seconds.

Figs. 30–32. Haemocyte meeting an obstruction during amoeba-like advance. Note probing activity of hyaline, filiform pseudopodia in two directions and advance with one at expense of other. Intervals between figures approximately 60 seconds.

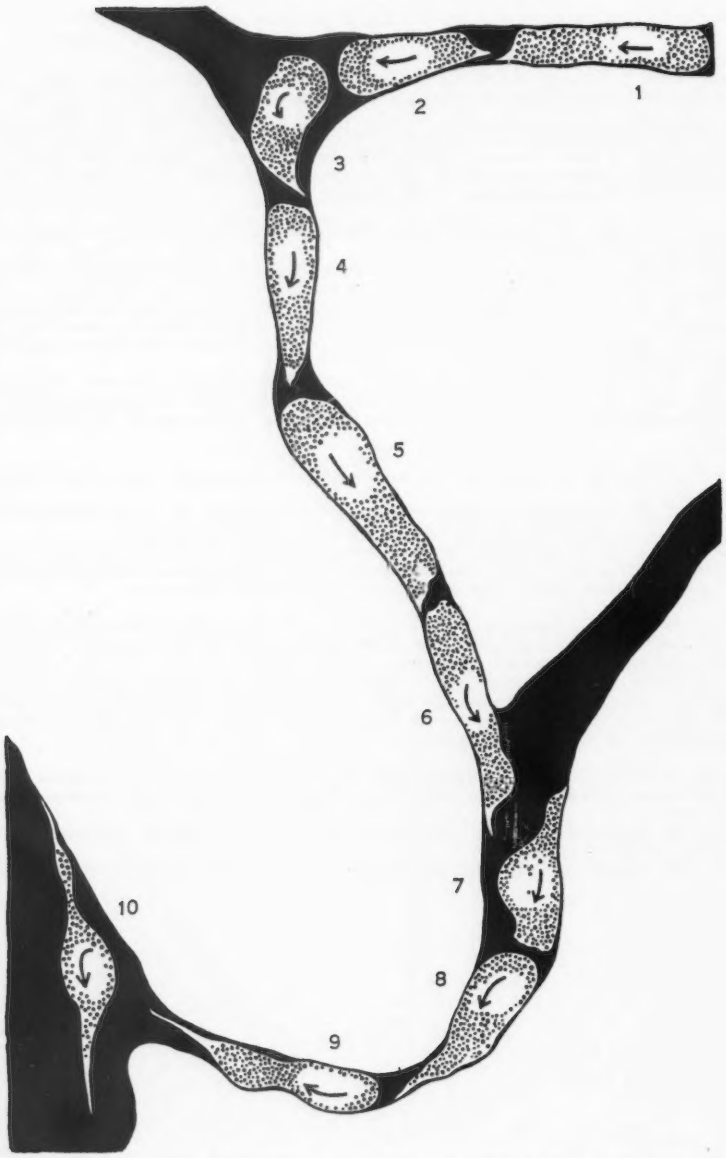
Figs. 33–36. Still photomicrographs and tracings of a haemocyte during rapid advance across a vein and in almost full contact with the vein wall, ×1000 (approx.). Intervals between figures approximately 30 seconds.

Figs. 37–43. Selected frames and tracings from time-lapse motion picture showing haemocyte during rapid extension and with the cell body free in the lumen of the vein, ×500 (approx.). The haemocyte adhered only at the "tail". Intervals between frames approximately 30 seconds but variable.

Figs. 44–47. Still photomicrographs and tracings of haemocyte forcing its way into and out of an extremely narrow passageway. Intervals between figures approximately 5 minutes.

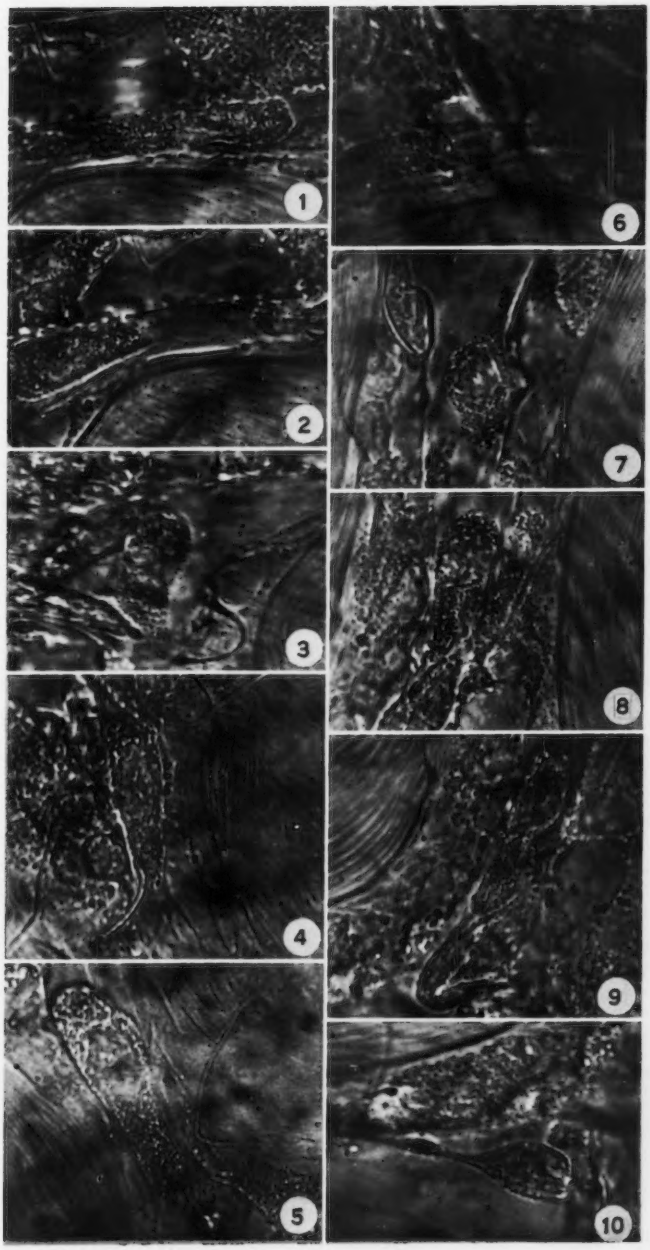
Fig. 48. Apparently "passive" haemocyte showing active terminal, hyaline, filiform extension, ×1000 (approx.).

Fig. 49. Fibrous "ghosts" of haemocytes in vein of a moribund roach, ×1000 (approx.).

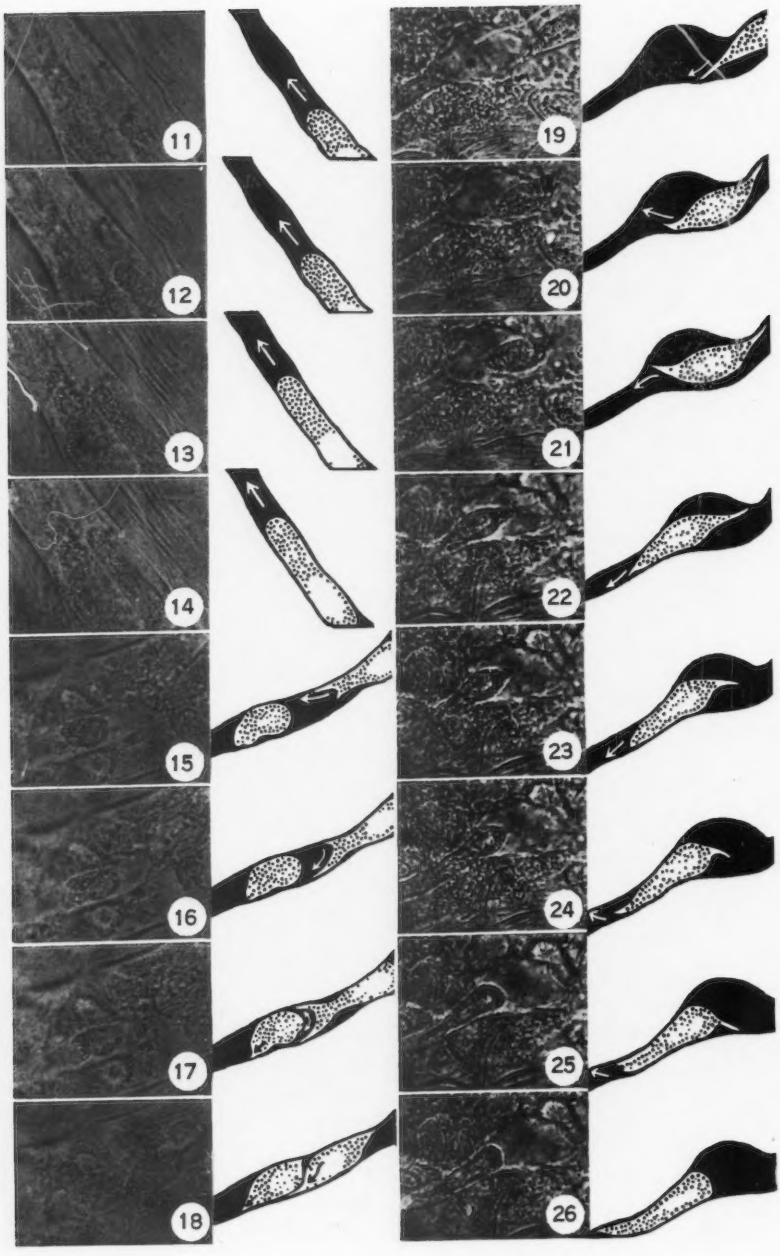


Tracings of photomicrographs from Figs. 1–10 in a diagram of the route travelled. Hyaline pseudopodia, not clearly visible in the photomicrographs, are the non-stippled areas. Nucleus also is not stippled. Arrows indicate the main direction of cytoplasmic flow.

PLATE I



Arnold—Can. J. Zool.



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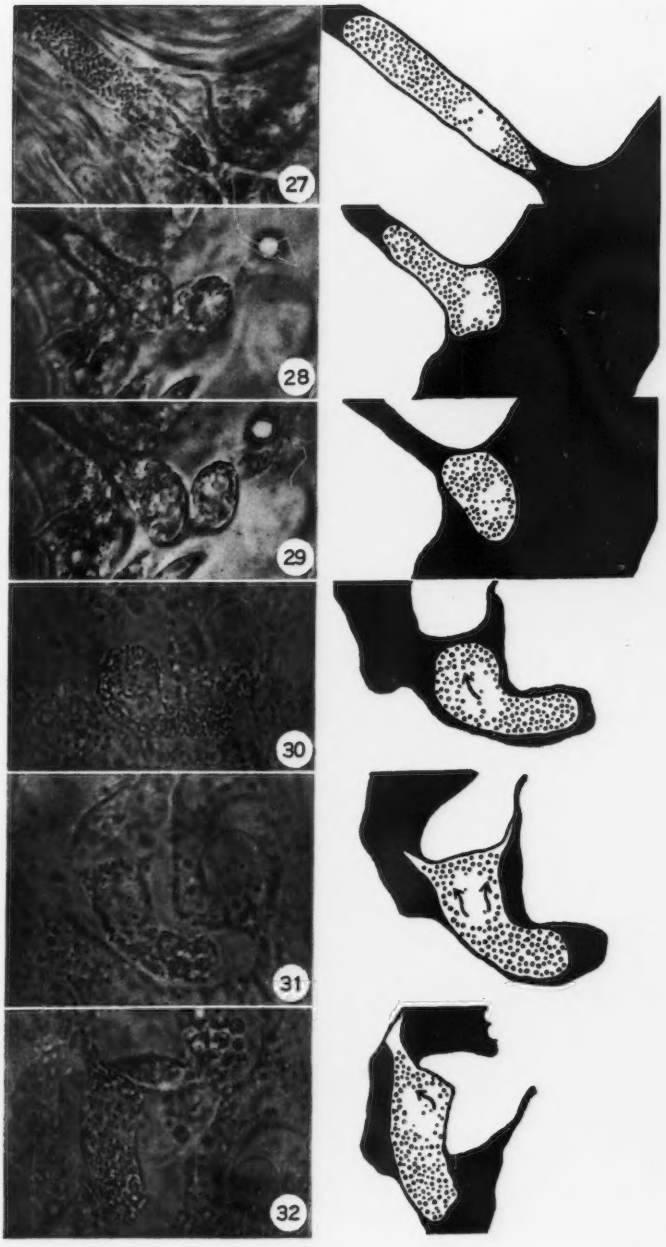

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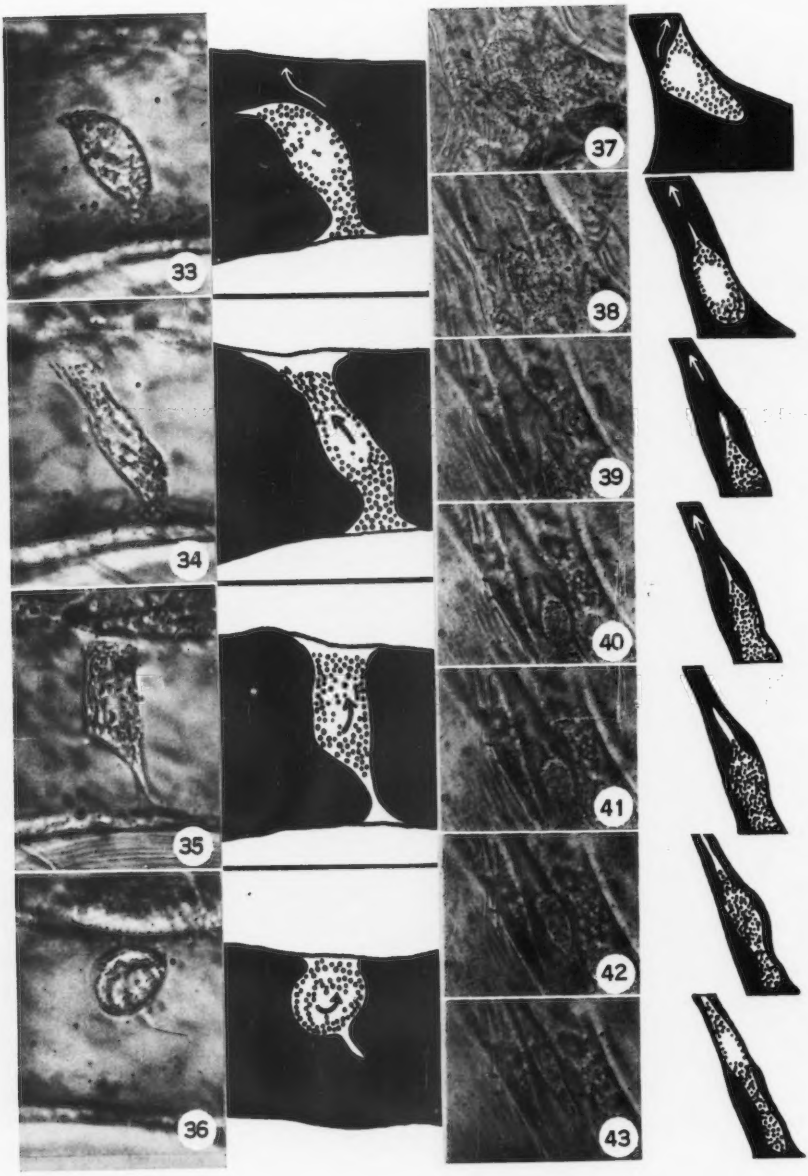

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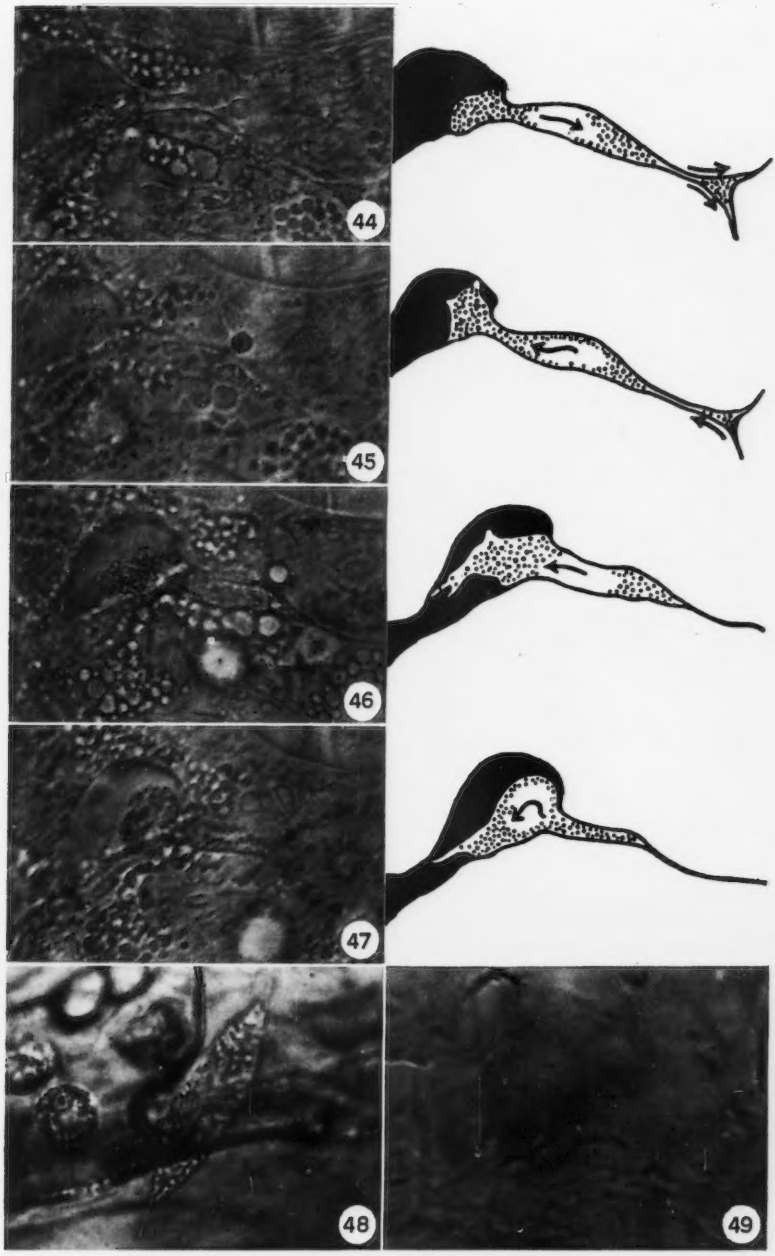

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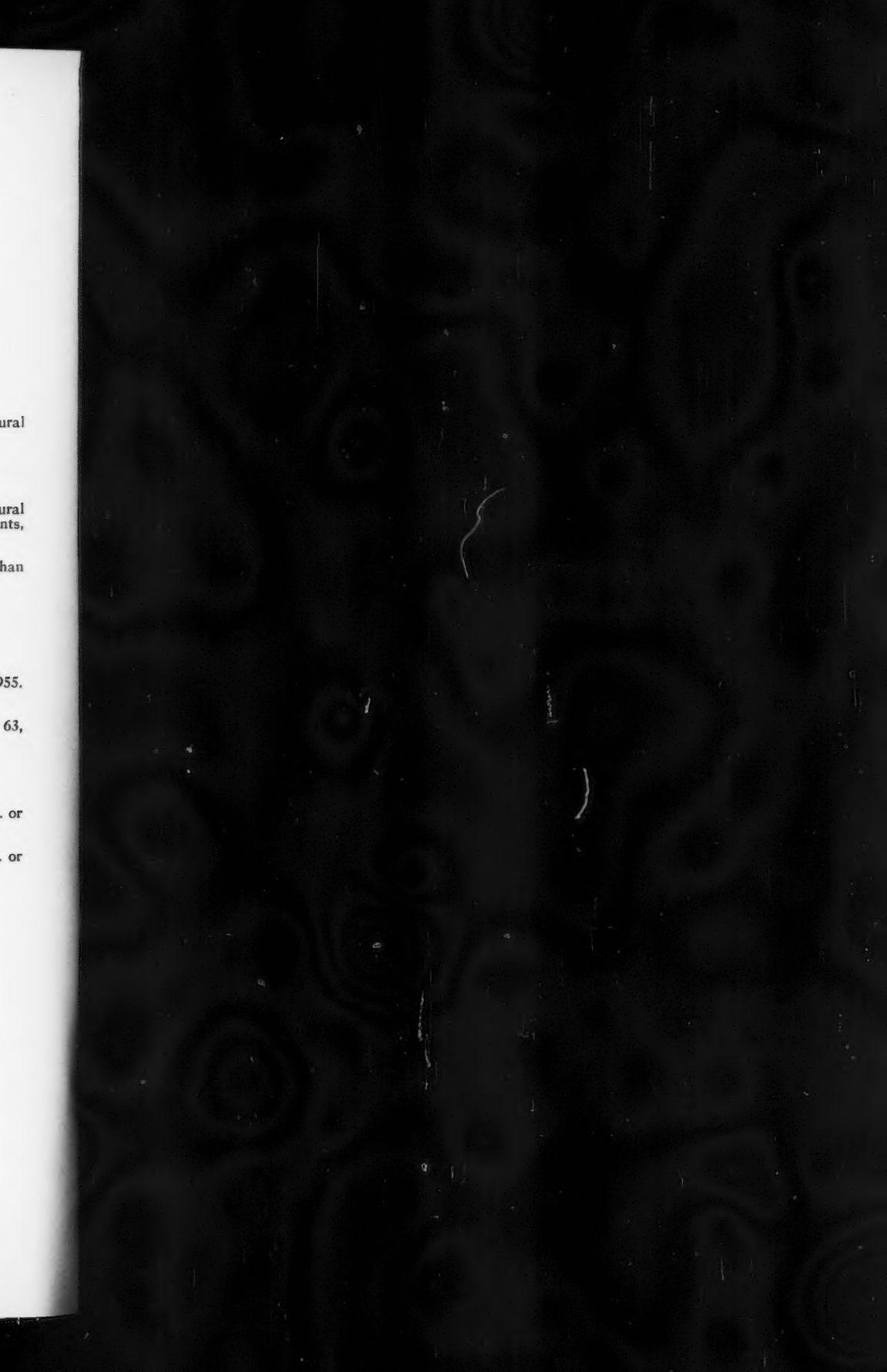

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